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NAALADASE INHIBITORS FOR TREATING RETINAL DISORDERS AND GLAUCOMA

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NAALADASE INHIBITORS FOR TREATING RETINAL DISORDERS AND GLAUCOMA

This application claims the benefit of U.S. Provisional Application No. 60/207,320 filed on May 30, 2000.

The present invention relates to pharmaceutical compositions and methods for treating glaucoma and retinal disorders using NAALADase inhibitors.

Glutamate has been implicated in the pathophysiology of various retinal disorders. For example, elevated vitreous glutamate levels have been detected in patients with proliferative diabetic retinopathy. Additionally, retinal detachment has been shown to induce rapid glutamatergic alterations in the neural retina. Abnormal formation and degradation of glutamate have also been observed in the retina during retinitis pigmentosa.

One source of glutamate is derived from the neuropeptide N-acetylated-aspartyl-glutamate (NAAG) through cleavage by N-acetylated-α-linked acidic dipeptidase (NAALADase), also known as prostate specific membrane antigen (PSM or PSMA) and human glutamate carboxypeptidase II (GCP II). Studies suggest that NAALADase inhibitors may block glutamate release pre-synaptically without interacting with post-synaptic glutamate receptors.

Glaucoma is a group of eye diseases characterized by optic disk excavation, retinal ganglion cell (RGC) loss

and visual field loss. Since elevated intraocular pressure (IOP) is a major risk factor for glaucoma, pharmacological and surgical treatments of glaucoma have been aimed at lowering IOP. The drugs currently used in treating glaucoma include miotics (e.g., pilocarpine, carbachol, and acetylcholinesterase inhibitors), sympathomimetics (e.g., epinephrine and dipivalylepinephrine), beta-blockers (e.g., timolol, betaxolol, carteolol, levobunolol and metipranolol), alpha-2 agonists (e.g., para-amino clonidine) and carbonic anhydrase inhibitors (e.g., acetazolamide, methazolamide and ethoxzolamide). Miotics and sympathomimetics are believed to lower IOP by increasing the outflow of aqueous humor, while beta-blockers, alpha-2 agonists and carbonic anhydrase inhibitors are believed to lower IOP by decreasing the formation of aqueous humor. All five types of drugs have potential side effects. Miotics, such as pilocarpine, can cause blurring of vision and other visual side effects. Sympathomimetics have a fairly high incidence of allergic or toxic reactions. Carbonic anhydrase inhibitors can cause serious side effects that may decrease patient compliance and/or require termination of drug therapy. At least one beta-blocker, timolol, has become associated with serious pulmonary side effects attributable to its effect on beta-2 receptors in pulmonary tissue. Notwithstanding the side effects, some cases of glaucoma continue to worsen despite IOP lowering. Thus, a need

exists for new glaucoma treatments.

SUMMARY OF THE INVENTION

The present inventors have surprisingly discovered that NAALADase inhibitors may be effective in slowing, if not preventing, retinal ganglion cell death associated with glaucoma. Thus, the present invention relates to a method for treating glaucoma comprising administering an effective amount of a NAALADase inhibitor to an animal or a mammal in need of such treatment.

The present invention further relates to a method for treating retinopathy, age-related macular degeneration or glaucoma comprising administering an effective amount of a NAALADase inhibitor to a mammal in need of such treatment.

The present invention also relates to a pharmaceutical composition comprising:

(i) an effective amount of a NAALADase inhibitor for treating retinopathy, age-related macular degeneration or glaucoma; and

(ii) a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a 27,000x magnified photograph of a retinal blood vessel from a control, non-diabetic rat.

FIG. 2 is a 27,000x magnified photograph of a retinal blood vessel from a diabetic rat after six months of treatment with a vehicle.

FIG. 3 is a 27,000x magnified photograph of a retinal blood vessel from a diabetic rat after six months of treatment with NAALADase inhibitor 2-(3-sulfanylpropyl)pentanedioic acid.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Alkyl" refers to a branched or unbranched saturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_1-C_9 alkyl is a straight or branched hydrocarbon chain containing 1 to 9 carbon atoms, and includes but is not limited to substituents such as methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert-butyl, n-pentyl, n-hexyl, and the like, unless otherwise indicated.

"Alkenyl" refers to a branched or unbranched unsaturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_2-C_9 alkenyl is a straight or branched hydrocarbon chain containing 2 to 9 carbon atoms having at least one double bond, and includes but is not limited to substituents such as ethenyl, propenyl, iso-propenyl, butenyl, iso-butenyl, tert-butenyl, n-pentenyl, n-hexenyl, and the like, unless otherwise indicated.

"Alkoxy" refers to the group -OR wherein R is alkyl as herein defined. Preferably, R is a branched or unbranched saturated hydrocarbon chain containing 1 to 9 carbon atoms.

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"Carbocycle" refers to a hydrocarbon, cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged. Examples include cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclopentene, cyclohexene, cycloheptene, cycloctene, benzyl, naphthene, anthracene, phenanthracene, biphenyl and pyrene.

"Aryl" refers to an aromatic, hydrocarbon cyclic moiety having one or more closed ring(s). Examples include, without limitation, phenyl, naphthyl, anthracenyl, phenanthracenyl, biphenyl and pyrenyl.

"Heterocycle" refers to a cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged, with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrolidine, pyrrole, thiazole, thiophene, piperidine, pyridine, isoxazolidine and isoxazole.

"Heteroaryl" refers to an aromatic, cyclic moiety having one or more closed ring(s) with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrole, thiophene, pyridine and isoxazole.

" Linking group" refers to a moiety that connects the terminal group with the benzene ring in the compounds of formula VI, without compromising with the pharmacological or biological activity of the overall compound.

"Metal binding group" refers to a functional group capable of interacting with metal ion(s), such as Co^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, or Al^{3+}. Common metal binding groups include amines (e.g. ethylenediamine), aldehydes, ketones, carboxylic acids (e.g. ethylenediaminetetraacetic acid (EDTA)), thiols, phosphorus derivatives and hydroxamic acids.

"Derivative" refers to a substance produced from another substance either directly or by modification or partial substitution.

"Effective amount" refers to the amount required to produce the desired effect.

"Therapeutically effective amount" refers to the amount required to treat glaucoma in an animal or a mammal.

"Halo" refers to at least one fluoro, chloro, bromo or iodo moiety.

"Isosteres" refer to elements, functional groups, substitutents, molecules or ions having different molecular formulae but exhibiting similar or identical physical properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have different molecular formulae. Typically, two isosteric molecules have similar or identical volumes and shapes. Ideally, isosteric compounds should be isomorphic and able to co-crystallize. Other physical properties that isosteric compounds usually share include boiling point,

density, viscosity and thermal conductivity. However, certain properties are usually different: dipolar moments, polarity, polarization, size and shape since the external orbitals may be hybridized differently. The term "isosteres" encompass "bioisosteres".

"Bioisosteres" are isosteres that, in addition to their physical similarities, share some common biological properties. Typically, bioisosteres interact with the same recognition site or produce broadly similar biological effects.

"Carboxylic acid isosteres" include without limitation direct derivatives such as hydroxamic acids, acyl-cyanamides and acylsulfonamides; planar acidic heterocycles such as tetrazoles, mercaptoazoles, sulfinylazoles, sulfonylazoles, isoxazoles, isothiazoles, hydroxythiadiazoles and hydroxychromes; and nonplanar sulfur- or phosphorus-derived acidic functions such as phosphinates, phosphonates, phosphonamides, sulphonates, sulphonamides, and acylsulphonamides.

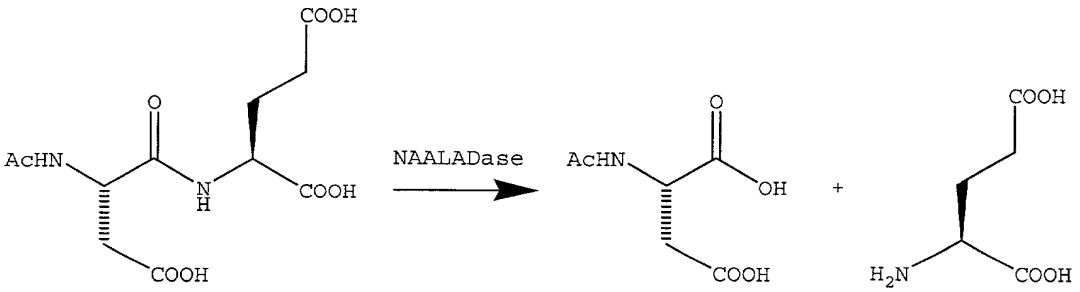
"Metabolite" refers to an intermediate or product resulting from metabolism.

"NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific, present in synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as

a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate. In addition, NAAG is an agonist at group II metabotropic glutamate receptors, specifically mGluR3 receptors; when attached to a moiety capable of inhibiting NAALADase, it is expected that metabotropic glutamate receptor ligands will provide potent and specific NAALADase inhibitors.

"NAALADase" refers to N-acetylated α-linked acidic dipeptidase, a membrane bound metallopeptidase that catabolizes NAAG to N-acetylaspartate ("NAA") and glutamate ("GLU"):

Catabolism of NAAG by NAALADase



NAALADase has been assigned to the M28 peptidase family and is also called prostate specific membrane antigen (PSM) or human glutamate carboxypeptidase II (GCP II), EC number 3.4.17.21. It is believed that NAALADase is a co-catalytic zinc/zinc metallopeptidase. NAALADase shows a high affinity for NAAG with a Km of 540 nM. If NAAG is a

bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

"Pharmaceutically acceptable carrier" refers to any carrier, diluent, excipient, wetting agent, buffering agent, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbent, preservative, surfactant, colorant, flavorant, or sweetener, preferably non-toxic, that would be suitable for use in a pharmaceutical composition.

"Pharmaceutically acceptable equivalent" includes, without limitation, pharmaceutically acceptable salts, hydrates, metabolites, prodrugs, and isosteres. Many pharmaceutically acceptable equivalents are expected to have the same or similar *in vitro* or *in vivo* activity as the inventive compounds.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds that possesses the desired pharmacological activity and that is neither biologically nor otherwise undesirable. The salt can be formed with acids that include without limitation acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycero-phosphate, hemisulfate, heptanoate, hexanoate, hydro-

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chloride hydrobromide, hydroiodide, 2-hydroxyethane-sulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

"Prodrug" refers to a derivative of the inventive compounds that undergoes biotransformation, such as metabolism, before exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the inventive compounds using methods known in the art, such as those described by *Burger's Medicinal Chemistry and*

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Drug Chemistry, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995).

"Inhibition," in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i, which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

wherein [E] is the concentration of the enzyme, [I] is the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase. "IC_{50}" is a related term used to define the concentration or amount of a compound that is required to cause a 50% inhibition of the target enzyme.

"NAALADase inhibitor" refers to any compound that inhibits NAALADase enzyme activity. Preferably, a

NAALADase inhibitor exhibits a K_i of less than 100 μM, more preferably less than 10 μM, and even more preferably less than 1 μM, as determined using any appropriate assay known in the art.

"Isomers" refer to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the arrangement or configuration of the atoms.

"Optical isomers" refer to enantiomers or diastereoisomers.

"Stereoisomers" are isomers that differ only in the arrangement of the atoms in space.

"Diastereoisomers" are stereoisomers that are not mirror images of each other. Diastereoisomers occur in compounds having two or more asymmetric carbon atoms; thus, such compounds have 2^n optical isomers, where *n* is the number of asymmetric carbon atoms

"Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other. Enantiomers result, for example, from the presence of one or more asymmetric carbon atom(s) in the compound (e.g., glyceraldehyde, lactic acid, sugars, tartaric acid, amino acids).

"Enantiomer-enriched" refers to a mixture in which one enantiomer predominates.

"Racemic mixture" means a mixture containing equal amounts of enantiomers.

"Non-racemic mixture" is a mixture containing unequal amounts of enantiomers.

"Animal" refers to a living organism having sensation and the power of voluntary movement, and which requires for its existence oxygen and organic food. Examples include, without limitation, members of the human, equine, porcine, bovine, murine, canine, or feline species. In the case of a human, an "animal" may also be referred to as a "patient".

"Mammal" refers to a warm-blooded vertebrate animal.

" Glaucoma" includes without limitation chronic (idiopathic) open-angle glaucomas (e.g., high-pressure, normal-pressure); pupillary block glaucomas (e.g., acute angle-closure, subacute angle-closure, chronic angle-closure, combined-mechanism); developmental glaucomas (e.g., congenital (infantile), juvenile, Anxenfeld-Rieger syndrome, Peters' anomaly, Aniridia); glaucomas associated with other ocular disorders (e.g., glaucomas associated with disorders of the corneal endothelium, iris, ciliary body, lens, retina, choroid and vitreous); glaucomas associated with elevated episcleral venous pressure (e.g., systemic diseases with associated elevated intraocular pressure and glaucoma, corticosteroid-induced glaucoma); glaucomas associated with inflammation and trauma (e.g., glaucomas associated with keratitis, episcleritis, scleritis, uveitis, ocular trauma and hemorrhage); glaucomas following intraocular surgery (e.g., ciliary block (malignant) glaucoma, glaucomas in aphakia and

pseudophakia, glaucomas associated with corneal surgery, glaucomas associated with vitreoretinal surgery).

"Treating" refers to:

(i) preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and/or

(iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

" Treating a retinal disorder" refers to:

(i) preventing a retinal disorder from occurring in an animal that may be predisposed to a retinal disorder but has not yet been diagnosed as having it;

(ii) inhibiting or slowing a retinal disorder, e.g. arresting its development; and/or

(iii) relieving a retinal disorder, e.g. causing its regression.

" Retinal disorder" refers to vascular retinopathy, for example, hypertensive retinopathy, diabetic retinopathy (nonproliferative or proliferative), central retinal artery occlusion, or central retinal vein occlusion; age-related macular degeneration; retinal detachment; or retinitis pigmentosa.

One of ordinary skill in the art would recognize that there are alternative nomenclatures, nosologies and

classification systems for the diseases, disorders and conditions defined above, and that such systems evolve with medical scientific progress.

Unless the context clearly dictates otherwise, the definitions of singular terms may be extrapolated to apply to their plural counterparts as they appear in the application; likewise, the definitions of plural terms may be extrapolated to apply to their singular counterparts as they appear in the application.

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METHODS OF THE PRESENT INVENTION

The present invention relates to a method for treating a retinal disorder comprising administering an effective amount of a NAALADase inhibitor to an animal or a mammal in need of such treatment.

The present invention further relates to a method for treating glaucoma comprising administering an effective amount of a NAALADase inhibitor to an animal or a mammal in need of such treatment.

PHARMACEUTICAL COMPOSITIONS OF THE PRESENT INVENTION

The present invention further relates to a pharmaceutical composition comprising:

(i) an effective amount of a NAALADase inhibitor for treating a retinal disorder or glaucoma; and

(ii) a pharmaceutically acceptable carrier.

NAALADASE INHIBITORS

NAALADase inhibitors that can be used in the inventive methods and pharmaceutical compositions include without limitation metallopeptidase inhibitors such as *o*-phenanthroline, metal chelators such as EGTA and EDTA, and peptide analogs such as quisqualic acid and ß-NAAG.

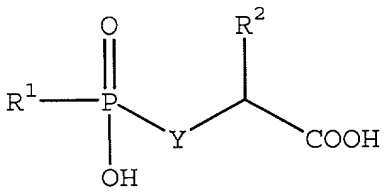
While the pathophysiology of retinal disorders or neuronal death in glaucoma is not well understood, there is evidence that it may involve glutamate excitotoxicity. Thus, a preferred NAALADase inhibitor is one that is capable of reducing or preventing glutamate-induced excitotoxicity, thereby reducing or preventing neuronal or retinal ganglion cell (RGC) damage or death resulting from such excitotoxicity. A particularly preferred NAALADase inhibitor for treating a retinal disorder is one that is capable of reducing or preventing RGC damage or death through a mechanism that does not modulate aqueous humor dynamics or intraocular pressure (IOP). While the foregoing attributes are preferred, the NAALADase inhibitors used in the inventive methods and pharmaceutical compositions may exert their therapeutic effects through other mechanisms of action.

Another preferred NAALADase inhibitor is an acid containing a metal binding group.

FORMULA I

Another preferred NAALADase inhibitor is a compound of formula I:

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I

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

Y is CR^3R^4, NR^5 or O;

R^1 is hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar, $COOR^6$, NR^6R^7 or OR^6, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s), preferably, independently selected from the group consisting of carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, $COOR^6$, NR^6R^7 and Ar;

R^2 is hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar, halo or carboxy, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s), preferably, independently selected from the group consisting of carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, NR^6R^7 and Ar;

R^3 and R^4 are independently hydrogen or C_1-C_3 alkyl;

R^5 is hydrogen or C_1-C_3 alkyl;

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R^6 and R^7 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl or Ar, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s), preferably, independently selected from the group consisting of carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s), preferably, independently selected from the group consisting of halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, carboxy and N^6R^7.

In one embodiment of formula I, Y is CH_2.

In another embodiment, R^2 is $-(CH_2)_2COOH$.

In a further embodiment, R^1 is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, benzyl, phenyl or OR^6, wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl, benzyl and phenyl are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl,

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halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, NR^6R^7, benzyl and phenyl.

Preferred compounds of formula I are selected from the group consisting of:

2-(phosphonomethyl)pentanedioic acid;

2-[[(2-carboxyethyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[(phenylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[((hydroxy)phenylmethyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

2-[(butylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(3-methylbenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(3-phenylpropylhydroxyphosphinyl)methyl]-pentanedioic acid;

2-[[(4-fluorophenyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[(phenylethylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(4-methylbenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(4-fluorobenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(4-methoxybenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

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2-[[(3-trifluoromethylbenzyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

2-[[4-trifluoromethylbenzyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

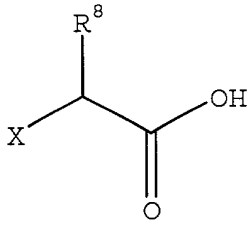
2-[[(2-fluorobenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(2,3,4,5,6-pentafluorobenzyl)hydroxy-phosphinyl]methyl]pentanedioic acid; and

enantiomers and pharmaceutically acceptable equivalents.

FORMULA II

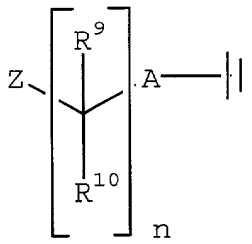
Another preferred NAALADase inhibitor is a compound of formula II



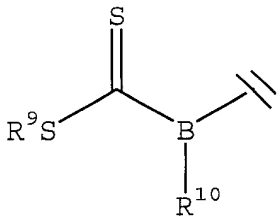
II

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

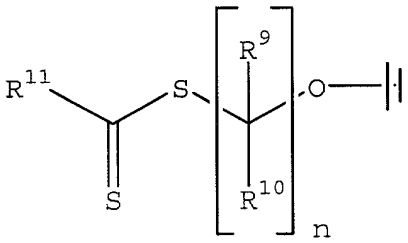
X is a moiety of formula III, IV or V



III



IV



V ;

Z is SH, SO_{3H}, SO_2H, SOH, $SO(NH)R^{12}$ or $S(NHR^{12})_2R^{13}$;

B is N or CR^{14};

A is O, S, $CR^{15}R^{16}$ or $(CR^{15}R^{16})_mS$;

m and n are independently 0, 1, 2, 3 or 4;

R^8, R^9, R^{10}, R^{11}, R^{12}, R^{14}, R^{15} and R^{16} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar^1, hydroxy, carboxy, carbonyl, amino, cyano, isocyano, nitro, sulfonyl, sulfoxy, thio, thiocarbonyl, thiocyano, formanilido, thioformamido, sulfhydryl, halo, haloalkyl, trifluoromethyl or oxy, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s); and

Ar^1 is a carbocyclic or heterocyclic moiety, which is unsubstituted or substituted with one or more substituent(s);

provided that when X is a moiety of formula III and A is O, then n is 2, 3 or 4; when X is a moiety of formula III and A is S, then n is 2, 3 or 4; and when X is a moiety of formula III and A is $(CR^{15}R^{16})_mS$, then n is 0, 2, 3 or 4.

In one embodiment of formula II, X is a moiety of formula III; n is 0, 1, 2 or 3; Z is SH, SO_3H, SO_2H, SOH or $S(NHR^{12})_2R^{13}$; and A is O, S or $CR^{15}R^{16}$.

In another embodiment, R^8 is $-(CH_2)_2COOH$.

In a further embodiment, Z is SH.

Preferred compounds of formula II are selected from the group consisting of:

2-(2-sulfanylethyl)pentanedioic acid;

3-(2-sulfanylethyl)-1,3,5-pentanetricarboxylic acid;

2-(2-sulfanylpropyl)pentanedioic acid;

2-(2-sulfanylbutyl)pentanedioic acid;

2-(2-sulfanyl-2-phenylethyl)pentanedioic acid;

2-(2-sulfanylhexyl)pentanedioic acid;

2-(2-sulfanyl-1-methylethyl)pentanedioic acid;

2-[1-(sulfanylmethyl)propyl]pentanedioic acid;

2-(3-sulfanylpentyl)pentanedioic acid;

2-(3-sulfanylpropyl)pentanedioic acid;

2-(3-sulfanyl-2-methylpropyl)pentanedioic acid;

2-(3-sulfanyl-2-phenylpropyl)pentanedioic acid;

2-(3-sulfanylbutyl)pentanedioic acid;

2-[3-sulfanyl-2-(phenylmethyl)propyl]pentanedioic acid;

2-[2-(sulfanylmethyl)butyl]pentanedioic acid;

2-[2-(sulfanylmethyl)pentyl]pentanedioic acid;

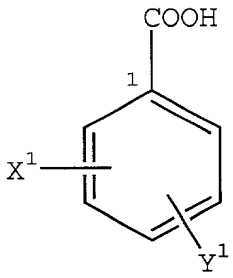
2-(3-sulfanyl-4-methylpentyl)pentanedioic acid; and

enantiomers and pharmaceutically acceptable equivalents.

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FORMULA VI

Another preferred NAALADase inhibitor is a compound of formula VI



VI

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^1 is -W-Z^1;

W is a bond or a linking group;

Z^1 is a terminal group; and

Y^1 is -COOH oriented *meta* or *para* relative to C-1.

Linking groups include, without limitation, divalent hydrocarbon chains, ethers, sulfides and amines, wherein the hydrocarbon chain, whether alone or part of the ether,

sulfide or amine, may be saturated or unsaturated, straight or branched, open or closed, unsubstituted or substituted with one or more substituent(s), preferably, independently selected from the group consisting of C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, hydroxy, carboxy, carbamido, carbamoyl, carbamyl, carbonyl, carbozoyl, amino, hydroxyamino, formamido, formyl, guanyl, cyano, cyanoamino, isocyano, isocyanato, diazo, azido, hydrazino, triazano, nitro, nitroso, isonitroso, nitrosamino, imino, nitrilo, isonitrilo, nitrosimino, oxo, C_1-C_6 alkylthio, sulfamino, sulfamoyl, sulfeno, sulfhydryl, sulfinyl, sulfo, sulfonyl, sulfoxy, thiocarboxy, thiocyano, isothiocyano, thioformamido, halo, haloalkyl, chlorosyl, chloryl, perchloryl, trifluoromethyl, iodosyl, iodyl, phosphino, phosphinyl, phospho, phosphono, arsino, selanyl, diselanyl, siloxy, silyl and silylene.

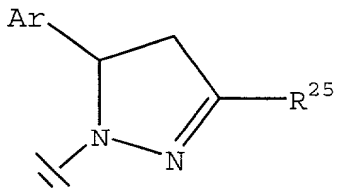
Preferably, W is a bond, $-(CR^{17}R^{18})_n-$, $-(CR^{17}R^{18})_nO(CR^{19}R^{20})_m-$, $-(CR^{17}R^{18})_nS(CR^{19}R^{20})_m-$ or $-(CR^{17}R^{18})_nNR^{21}(CR^{19}R^{20})_m-$, wherein m and n are independently 0-9, and R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_6-C_{14} aryl, heteroaryl, C_6-C_{14} carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_6 alkoxy, and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy are independently unsubstituted or substituted with one or more substituent(s). More preferably, R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are each hydrogen and the total number of carbon atoms in

W is 2-6.

Preferably, Z^1 is a metal binding group. More preferably, Z^1 is -COOH, $-COR^{22}$, $-OR^{22}$, $-CF_3$, -CN, -F, -Cl, -Br, -I, -NO, $-NO_2$, $-C(O)(NR^{22}OR^{23})$, $-C(O)(NR^{22}PO_3H_2)$, $-C(O)(NR^{22}R^{23})$, =NOH, $-NR^{22}(P(O)(R^{23})OH)$, $=NR^{22}$, $-N=NR^{22}$, $-N(R^{22})CN$, $-NR^{22}(CR^{23}R^{24})_pCOOH$, $-NR^{22}(CO)NR^{23}R^{24}$, $-NR^{22}(COOR^{23})$, $-NR^{22}(CO)R^{23}$, $-NR^{22}(OR^{23})$, $-NR^{22}R^{23}$, $-NR^{22}(SO_2R^{23})$, $-O(CO)R^{22}$, $-OR^{22}$, $-SO_2(OR^{22})$, $-SO_2(NR^{22}R^{23})$, $-SO_2R^{22}$, $-SO_3R^{22}$, $-SNR^{22}(OR^{23})$, $-S(NR^{22}R^{23})$, $-SR^{22}$, $-SSR^{22}$, $-P(O)(OH)OR^{22}$, $-P(O)(OH)R^{22}$ or $-PR^{22}R^{23}$, wherein p is 0-6, and R^{22}, R^{23} and R^{24} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_6-C_{14} aryl, heteroaryl, C_6-C_{14} carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_9 alkoxy, and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy are independently unsubstituted or substituted with one or more substituent(s). Even more preferably, Z^1 is $-NH(CR^{23}R^{24})_pCOOH$, $-PO(OH)OR^{22}$, $-PO(OH)R^{22}$, $-NR^{22}(P(O)(R^{23})OH)$, $-CON(R^{22})(OH)$ or -SH.

In one embodiment of formula VI:

X^1 is $-(CR^{17}R^{18})_nNH(CR^{19}R^{20})_mCOOH$, $-PO(OH)OR^{22}$, $-(CR^{17}R^{18})_nP(O)(OH)R^{22}$, $-NH-(CR^{19}R^{20})_m$-heteroaryl, $-NH(P(O)(R^{23})OH)$, $-(CR^{17}R^{18})_nNH(P(O)(OH)R^{23})$, $-CON(R^{22})(OH)$ $-(CR^{17}CR^{18})_nCON(R^{22})(OH)$, $-(CR^{17}R^{18})_nSH$ or $-O(CR^{19}R^{20})_mSH$, $-SO_2NH$-aryl, $-N(C=O)-CH_2(C=O)$-aryl, $-SO_2NH$-aryl, $-N(C=O)-CH_2(C=O)$-aryl, -O-aryl wherein aryl in -O-aryl is substituted by at least one of nitro, carboxy or



wherein X^1 is oriented *meta* or *para* relative to C-1;

m and n are independently 1-3, provided that when X^1 is $-O(CR^{19}R^{20})_mSH$, then m is 2 or 3;

R^{17}, R^{18}, R^{19}, R^{20}, R^{22}, R^{23} and R^{25} are independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_6 alkoxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy are independently unsubstituted or substituted with one or more substituent(s); and

Y^1 is -COOH oriented *meta* or *para* relative to C-1.

Preferably, when X is $-PO(OH)OR^{22}$ or $-(CR^{17}R^{18})_nP(O)(OH)OR^{22}$, then R^{22} is not H or methyl; when X is $-NH(P(O)(R^{23})OH$ or $-(CR^{17}R^{18})_nNH(P(O)(OH)R^{23})$, then R^{23} is not benzyl unsubstituted or substituted with amino; and when X is $-CON(R^{22})(OH)$, then R^{22} is not H or methyl

In another embodiment of formula VI, X^1 is oriented *meta* relative to C-1, and Y^1 is oriented *ortho* relative to X^1 and *para* relative to C-1. Preferably, W is a bond, $-(CH_2)_n-NH-(CH_2)_m-$ or $-(CH_2)_n-$; m is 1-3; n is 0-3; and Z^1 is $-CO_2H$, $-NO_2$, $-NH_2$, $-SO_3H$, halo, C_5-C_6 heteroaryl, carboxyphenylthio, or mono- or di-carboxyphenylsulfonyl.

Examples of this embodiment are:

2-[(4-carboxyphenyl)sulfonyl]-1,4-benzene-dicarboxylic acid;

2-[(2,5-dicarboxyphenyl)sulfonyl]-1,4-benzene-dicarboxylic acid;

1,2,4-benzenetricarboxylic acid;

2-[(2-carboxyphenyl)thio]-1,4-benzenedicarboxylic acid;

2-nitro-1,4-benzenedicarboxylic acid;

2-bromo-1,4-benzenedicarboxylic acid;

2-amino-1,4-benzenedicarboxylic acid;

2-sulfoterephthalic acid, monosodium salt;

2-carboxymethyl-1,4-benzenedicarboxylic acid;

2-[(2-furanylmethyl)-amino]-1,4-benzenedicarboxylic acid;

2-[(carboxymethyl)amino]-1,4-benzenedicarboxylic acid; and

enantiomers and pharmaceutically acceptable equivalents.

In another embodiment of formula VI, X^1 is oriented *ortho* relative to C-1, and Y^1 is oriented *para* relative to X^1 and *meta* relative to C-1. Preferably, (1) when W is a bond, then Z^1 is $-CO_2H$, -OH, $-NO_2$, $-C(O)(NHR^{23})$, $-SR^{23}$, $-COR^{23}$ or $-NH(CH_2R^{23})$, and R^{23} is an aryl or a heteroaryl wherein said aryl and heteroaryl are independently unsubstituted

or substituted with one or more alkyl, nitro or carboxy group(s); and (2) when W is $-(CH_2)_n-$ and n is 1-3, then Z^1 is -SH.

Examples of this embodiment are:

4-(4-nitrobenzoyl)-1,3-benzenedicarboxylic acid;

4-[4-(2,4-dicarboxybenzoyl)phenoxy]-1,2-benzene-dicarboxylic acid;

4-[[(2,4,6-trimethylphenyl)amino]carbonyl]-1,3-benzenedicarboxylic acid;

4-nitro-1,3-benzenedicarboxylic acid;

4-[(1-naphthalenylamino)-carbonyl]-1,3-benzene-dicarboxylic acid;

1,2,4-benzenetricarboxylic acid;

4-[(2-carboxyphenyl)thio]-1,3-benzenedicarboxylic acid;

4-[3-[[3-(2,4-dicarboxyphenoxy)propyl]dithio]-propoxy]-1,3-benzenedicarboxylic acid;

4-hydroxy-1,3-benzenedicarboxylic acid;

4-[(2-furanylmethyl)amino]-1,3-benzenedicarboxylic acid;

4-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid; and enantiomers and pharmaceutically acceptable equivalents.

In another embodiment of formula VI, X^1 is oriented *meta* relative to C-1, and Y^1 is oriented *meta* relative to X^1 and *meta* relative to C-1. Preferably, (1) when W is a bond, $-(CH_2)_n-$ or $-O(CH_2)_m-$ and m and n are independently 0-3, then Z^1 is $-SO_3H$, $-NO_2$, $-NH_2$, $-CO_2H$, -OH, $-PO_3H$, -CO(NHOH)

or -SH; (2) when W is $-(CH_2)_nNH(CH_2)_m-$ and m and n are independently 0-3, then Z^1 is $-CO_2H$ or C_5-C_6 heteroaryl; and (3) when W is a bond, then Z^1 is either (a) a heteroaryl that is unsubstituted or substituted with an aryl that is unsubstituted or substituted with one or more C_1-C_3 alkyl, halo, nitro or hydroxy group(s), or (b) $-SO_2(NHR^{24})$ or $-NH(COR^{24})$, wherein R^{24} is an aryl that is unsubstituted or substituted with one or more nitro, amino, halo or hydroxy group(s).

Examples of this embodiment are:

5-[4,5-dihydro-5-(4-hydroxyphenyl)-3-phenyl-1H-pyrazol-1-yl]-1,3-benzenedicarboxylic acid;

5-(4,5-dihydro-3-methyl-5-phenyl-1H-pyrazol-1-yl)-1,3-benzenedicarboxylic acid;

5-[[(4-chloro-3-nitrophenyl)amino]sulfonyl]-1,3-benzenedicarboxylic acid;

5-[[[4-chloro-3-[[3-(2-methoxyphenyl)-1,3-dioxopropyl]amino]phenyl]amino]sulfonyl-1,3-benzenedicarboxylic acid;

5-[[3-[4-(acetylamino)phenyl]-1,3-dioxopropyl]amino]-1,3-benzenedicarboxylic acid;

5-acetylamino-1,3-benzenedicarboxylic acid;

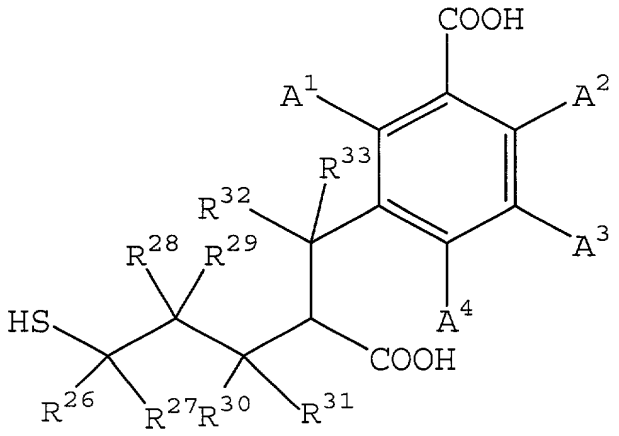
5-[[(1-hydroxy-2-naphthalenyl)carbonyl]-methylamino]-1,3-benzenedicarboxylic acid;

5-(4-carboxy-2-nitrophenoxy)-1,3-benzenedicarboxylic acid;

enantiomers and pharmaceutically acceptable equivalents.

FORMULA VII

Another preferred NAALADase inhibitor is a compound of formula VII



VII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or C_1-C_3 alkyl;

A^1, A^2, A^3 and A^4 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo, nitro, phenyl, phenoxy, benzyl, benzyloxy or -COOH, or any adjacent two of A^2, A^3 and A^4 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

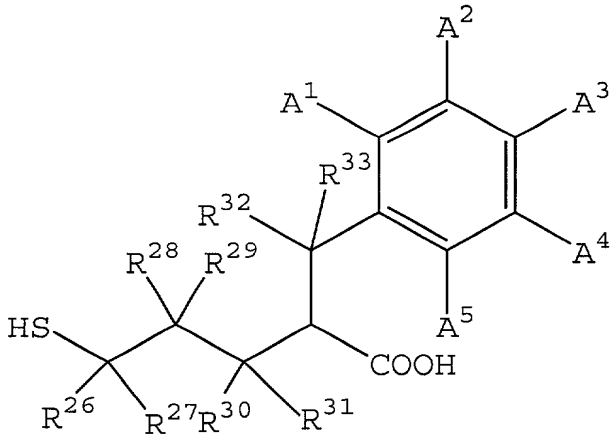
In one embodiment, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or methyl; and A^1, A^2, A^3 and A^4

are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, halo, nitro, phenyl, phenoxy, benzyloxy, nitro or -COOH.

In another embodiment, any adjacent two of A^2, A^3 and A^4 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

FORMULA VIII

Another preferred NAALADase inhibitor is a compound of formula VIII



VIII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or C_1-C_3 alkyl; and

A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_3 perhaloalkyl, phenyl, phenoxy, benzyl, benzyloxy, hydroxy, halo, cyano, nitro, $-SO_2R^{34}$, $-(C=O)NR^{34}R^{35}$, $-(C=O)NR^{34}(CH_2)_nCOOH$, $-NR^{34}(C=O)R^{35}$, $-(CH_2)_nCOOH$

5-sulfo-1,3-benzenedicarboxylic acid;

5-nitro-1,3-benzenedicarboxylic acid;

5-amino-1,3-benzenedicarboxylic acid;

1,3,5-benzenetricarboxylic acid;

5-[[(3-amino-4-chlorophenyl)amino]sulfonyl]-1,3-benzenedicarboxylic acid;

5-(3-mercaptopropoxy)-1,3-benzenedicarboxylic acid;

5-hydroxy-1,3-benzenedicarboxylic acid;

5-(2-mercaptoethoxy)-1,3-benzenedicarboxylic acid;

5-[(hydroxyamino)carbonyl]-1,3-benzenedicarboxylic acid;

5-phosphono-1,3-benzenedicarboxylic acid;

5-mercaptomethyl-1,3-benzenedicarboxylic acid;

5-phosphonomethyl-1,3-benzenedicarboxylic acid;

5-[[(carboxymethyl)amino]-methyl]-1,3-benzene-dicarboxylic acid;

5-[(carboxymethyl)amino]-1,3-benzenedicarboxylic acid;

5-[[(2-furanylmethyl)amino]-methyl]-1,3-benzene-dicarboxylic acid;

5-[2-(hydroxyamino)-2-oxoethyl]-1,3-benzene-dicarboxylic acid;

5-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid; and

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or -COOH, or any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

R^{34} and R^{35} are independently hydrogen, C_1-C_6 alkyl, phenyl or benzyl; and

n is 1-3.

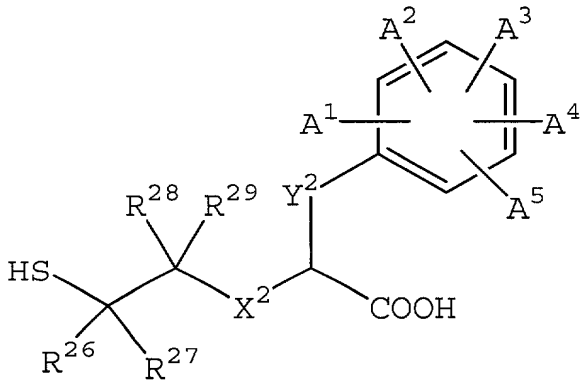
Preferably, if A^1, A^3 and A^5 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo, nitro, phenyl, phenoxy, benzyl, benzyloxy or -COOH, then neither A^2 nor A^4 are -COOH; and if any adjacent two of A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s), then A^2 is not -COOH.

In one embodiment, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are each hydrogen; A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, C_1-C_2 perhaloalkyl, phenyl, phenoxy, hydroxy, halo, cyano, nitro, $-SO_2R^{34}$, $-(C=O)NR^{34}R^{35}$, $-(C=O)NR^{34}(CH_2)COOH$, $-NR^{34}(C=O)R^{35}$ or $-(CH_2)COOH$; and R^{34} and R^{35} are independently hydrogen, methyl or benzyl.

In another embodiment, any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

FORMULA IX

Another preferred NAALADase inhibitor is a compound of formula IX



IX

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^2 and Y^2 are independently $-CR^{30}R^{31}-$, -O-, -S- or $-NR^{30}-$, provided that at least one of X^2 and Y^2 is/are $-CR^{30}R^{31}-$;

A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, $-COOR^{34}$, $-COR^{34}$, $-NR^{34}R^{35}$, $-SR^{34}$, $-SOR^{34}$, $-SO_2R^{34}$, $-SO_2(OR^{34})$, $-(C=O)NR^{34}R^{35}$, $-(C=O)NR^{34}(CH_2)_nCOOH$, $-NR^{34}(C=O)R^{35}$ or $-(CH_2)_nCOOH$, or any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3; and

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R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{34} and R^{35} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy, benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

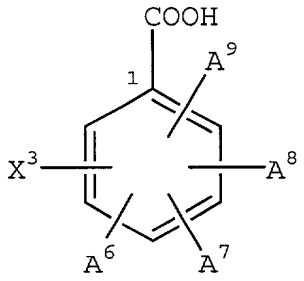
Preferably, if A^1, A^2 and A^3 are each hydrogen, and A^4 and A^5 are each -COOH, then A^4 is *ortho* to A^5; and if Y^3 is $-CR^{30}R^{31}-$, then at least one of A^1, A^2, A^3, A^4 and A^5 is/are independently phenoxy, benzyloxy, aryl, heteroaryl, carbocycle or heterocycle that is substituted with one or more substituent(s).

In one embodiment, Y^2 is -O-, -S- or $-NR^{30}-$; A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, hydroxy, halo, -COOH, $-COR^{34}$, $-NR^{34}(C=O)R^{35}$ or $-(CH_2)COOH$; and R^{34} and R^{35} are independently hydrogen or methyl.

In another embodiment, Y^2 is $-CR^{30}R^{31}-$; A^1, A^2, A^3 and A^4 are each hydrogen; and A^5 is phenoxy, benzyloxy, aryl, heteroaryl, carbocycle or heterocycle, wherein said phenoxy and benzyloxy are substituted with -COOH, and said aryl, heteroaryl, carbocycle and heterocycle are independently substituted with one or more substituent(s) selected from the group consisting of cyano and -COOH.

FORMULA X

Another preferred NAALADase inhibitor is a compound of formula X



X

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^3 is $-(CR^{36}R^{37})_nSH$, $-O(CR^{36}R^{37})_2SH$, $-S(CR^{36}R^{37})_2SH$ or $-NR(CR^{36}R^{37})_2SH$;

n is 1-3; and

R, R^{36}, R^{37}, A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

Preferred compounds of formula X are selected from the group consisting of:

3-(2-mercaptoethyl)-benzoic acid;

3-(mercaptomethyl)-benzoic acid;

2-(mercaptomethyl)-benzoic acid;

5-hydroxy-2-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-benzoic acid;

5-[(4-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-5-(phenylmethoxy)-benzoic acid;

2-(carboxymethoxy)-6-(2-mercaptoethyl)-benzoic acid;

5-[(3-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-6-(phenylmethoxy)-benzoic acid;

2-[(2-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;

2-(3,3-dimethylbutoxy)-6-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-6-(2-phenylethoxy)-benzoic acid;

2-[(2-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[[3-carboxy-5-(1,1-dimethylethyl)phenyl]methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-6-phenoxy-benzoic acid;

2-(2-mercaptoethyl)-6-phenylamino-benzoic acid;

2-(2-mercaptoethyl)-6-(phenylthio)-benzoic acid;

5'-(1,1-dimethylethyl)-3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;

3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,4'-dicarboxylic acid;

2-[(4-carboxy-2-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(4-carboxy-3-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(2-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(3-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(4-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-(biphenyl-2-ylmethoxy)-6-(2-mercaptoethyl)-benzoic acid;

2-[(3-bromo-5-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(2-bromo-5-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-6-[(4-methoxyphenyl)methoxy]-benzoic acid;

2-(2-mercaptoethyl)-6-[(4-methylphenyl)methoxy]-benzoic acid;

2-[(4-bromo-3-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(2-carboxy-5-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

5-(mercaptomethyl)-2-(2-phenylethoxy)-benzoic acid;

2-bromo-5-(mercaptomethyl)-benzoic acid;

4-(mercaptomethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;

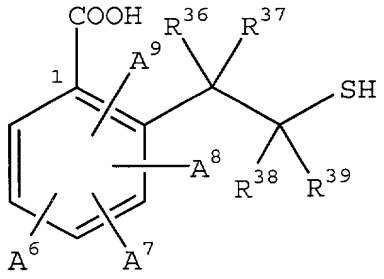
5-(mercaptomethyl)-2-(phenylmethoxy)-benzoic acid; and

4-bromo-3-(mercaptomethyl)-benzoic acid; and

enantiomers and pharmaceutically acceptable equivalents.

FORMULA XI

Another preferred NAALADase inhibitor is a compound of formula XI



XI

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{37}, R^{38}, R^{39} and R^{40} are independently hydrogen or C_1-C_3 alkyl; and

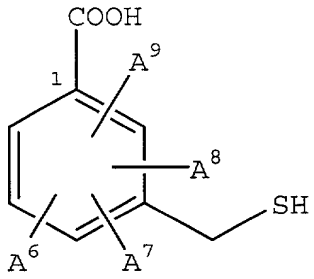
A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9

alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

In one embodiment, R^{36}, R^{37}, R^{38} and R^{39}, A^7, A^8 and A^9 are each hydrogen; A^6 is hydrogen, $-(CH_2)_n-W^1$, or $-Y^3-(CH_2)_n-W^1$; n is 0-3; Y^3 is O, S or NR^{40}; R^{40} is hydrogen or C_1-C_4 alkyl; W^1 is C_1-C_6 alkyl or phenyl, wherein W^1 is unsubstituted or substituted with C_1-C_4 alkyl, C_1-C_4 alkoxy, carboxy or halo.

FORMULA XII

Another preferred NAALADase inhibitor is a compound of formula XII



XII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9

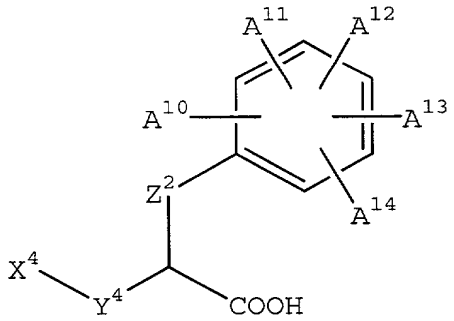
alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

Preferably, at least one of A^6, A^7, A^8 and A^9 is/are not hydrogen; and if A^6 is chloro, fluoro, amino or thiomethyl, then at least one of A^7, A^8 and A^9 is/are not hydrogen.

In one embodiment, A^7, A^8 and A^9 are each hydrogen; A^6 is $-(CH_2)_n-Ar^2$ or $-Y^3-(CH_2)_n-Ar^2$; n is 0-3; Y^3 is O, S or NR^{41}; R^{41} is hydrogen or C_1-C_4 alkyl; and Ar^2 is phenyl, wherein Ar^2 is unsubstituted or substituted with C_1-C_4 alkyl, carboxy or halo.

FORMULA XIII

Another preferred NAALADase inhibitor is a compound of formula XIII



XIII

or an enantiomer pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is -(CO)NHOH or -N(OH)COH;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from the group consisting of oxygen, sulfur and nitrogen;

Z^2 is $-CR^{41}R^{42}-$, $-NR^{41}-$, -O- or -S-;

A^{10}, A^{11}, A^{12}, A^{13} and A^{14} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, $-COOR^{43}$, $-COR^{43}$, $-NR^{43}R^{44}$, $-SR^{43}$, $-SOR^{43}$, $-SO_2R^{43}$, $-SO_2(OR^{43})$, $-(CO)NR^{43}R^{43}$, $-(CO)NR^{43}(CH_2)_nCOOH$, $-NR^{43}(CO)R^{44}$ or $-(CH_2)_nCOOH$, or any adjacent two of A^{10}, A^{11}, A^{12} and A^{13} form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3;

R^{41}, R^{42}, R^{43} and R^{44} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and

said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy, benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

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In one embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, $-O-$, $-S-$ or $-SO_2-$; p and q are independently 0-4, provided that when q is 0 and W^2 is $-NR^{49}-$, $-O-$, $-S-$ or $-SO_2-$, then Z^2 is $-CR^{41}R^{42}-$; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); and A^{10}, A^{11} and A^{12} are each hydrogen.

In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$; p is 0-4; q is 0; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are each hydrogen; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, $-COOR^{43}$, C_1-C_4 alkyl, C_2-C_4 alkenyl or C_2-C_4 alkynyl; and A^{14} is $-COOR^{43}$.

In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-S-$; p and q are independently 1-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl or phenyl; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, phenyl, benzyl, phenoxy, benzyloxy or halo, wherein said alkyl, alkenyl, alkynyl, phenyl, benzyl, phenoxy and benzyloxy are independently unsubstituted or substituted with carobxy; and A^{14} is -COOH.

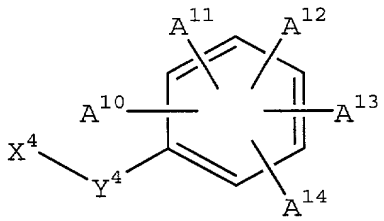
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In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4, provided that when q is 0 and W^2 is $-NR^{49}-$, -O-, -S- or $-SO_2-$, then Z^2 is $-CR^{41}R^{42}-$; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen; and A^{14} is benzyl or carboxybenzyl.

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FORMULA XIV

Another preferred NAALADase inhibitor is a compound of formula XIV



XIV

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is -(CO)NHOH or -N(OH)COH;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from the group consisting of oxygen, sulfur and nitrogen;

A^{10}, A^{11}, A^{12}, A^{13} and A^{14} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, $-COOR^{43}$, $-COR^{43}$, $-NR^{43}R^{44}$, $-SR^{43}$, $-SOR^{43}$, $-SO_2R^{43}$, $-SO_2(OR^{43})$, $-(CO)NR^{43}R^{44}$, $-(CO)NR^{43}(CH_2)_nCOOH$, $-NR^{43}(CO)R^{44}$ or $-(CH_2)_nCOOH$, or any adjacent two of A^{10}, A^{11}, A^{12} and A^{13} form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3;

R^{43} and R^{44} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and

said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy, benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

In one embodiment, Y^4 is a bond or $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9

alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); and A^{10}, A^{11} and A^{12} are each hydrogen.

In another embodiment, Y^4 is a bond; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydroxy, phenoxy, benzyloxy, $-COOR^{43}$ or $-(CO)NHR^{44}$; A^{14} is $-COOR^{43}$; R^{43} is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl or C_2-C_4 alkynyl; R^{44} is benzyl; and said benzyl, phenoxy and benzyloxy are independently unsubstituted or substituted with $-COOR^{43}$.

In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is -O- or -S-; R^{45}, R^{46}, R^{47} and R^{48} are each hydrogen; A^{10}, A^{11} and A^{12} are each hydrogen; and A^{13} is hydrogen, -COOH, phenyl or benzyloxy, wherein said phenyl and benzyloxy are independently unsubstituted or substituted with $-COOR^{43}$; and A^{14} is $-COOR^{43}$.

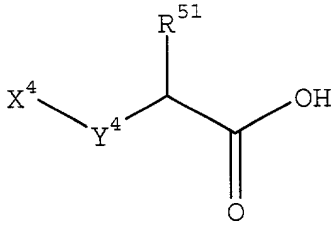
In another embodiment, Y^4 is a bond or $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido,

thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, nitro or C_1-C_4 alkoxy; A^{14} is hydroxy, phenoxy, benzyloxy, benzoyl or C_1-C_4 alkoxy, wherein said phenoxy, benzyloxy, benzoyl and alkoxy are independently unsubstituted or substituted with one or more substituent(s).

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FORMULA XV

Another preferred NAALADase inhibitor is a compound of formula XV



XV

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is -(CO)NHOH or -N(OH)COH;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from the group consisting of oxygen, sulfur and nitrogen; and

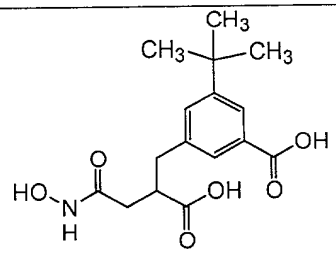
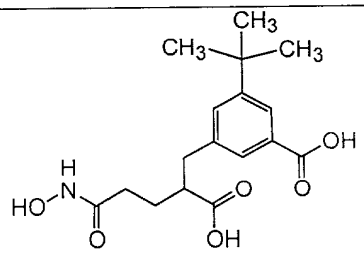
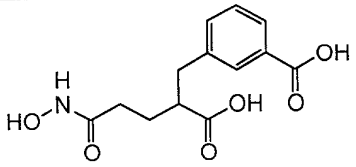
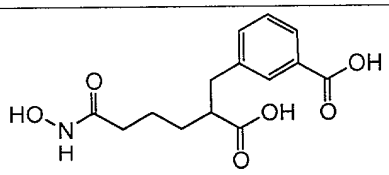
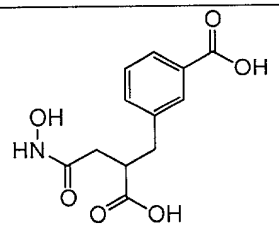
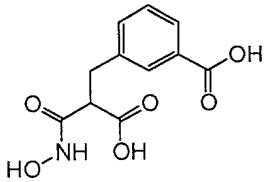
R^{51} is hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_1-C_9 alkoxy or C_2-C_9 alkenoxy, wherein said alkyl, alkenyl, alkynyl, alkoxy and alkenoxy are independently unsubstituted or substituted with one or more substituent(s); provided that when Y is methylene, amine or oxygen, then R^{51} is not carboxyethyl.

In one embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, $-O-$, $-S-$ or $-SO_2-$; p and q are independently 0-4; and R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

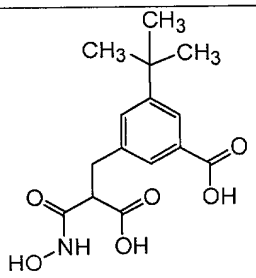
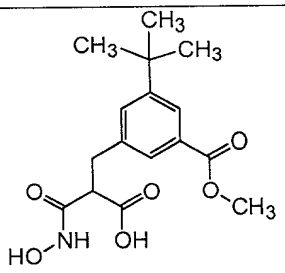
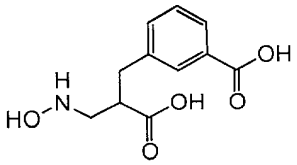
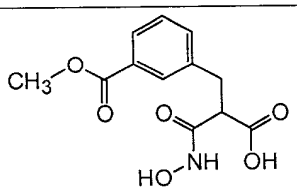
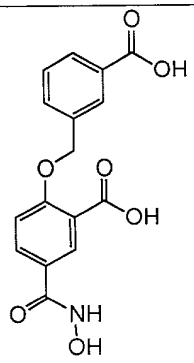
In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$ or $-S-$; p is 0-1; q is 0-3; and R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are each hydrogen.

Representative compounds of formulas XIII-XV are set forth below in TABLE I.

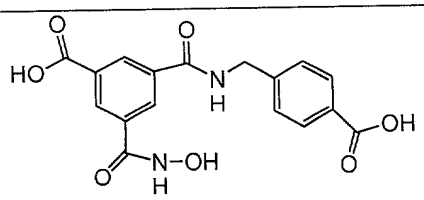
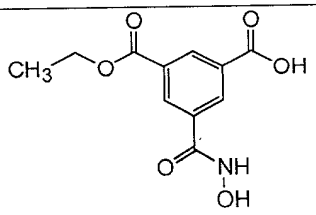
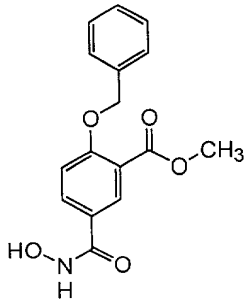
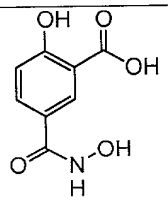
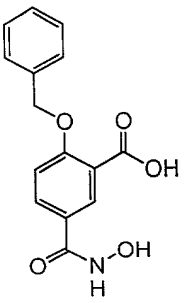
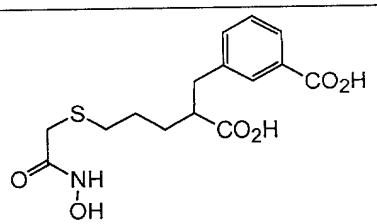
TABLE I

Structure	Name
	3-*tert*-Butyl-5-(2-carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid
	3-(2-Carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoyl-pentyl)-benzoic acid
	3-(2-Carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid
	3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid

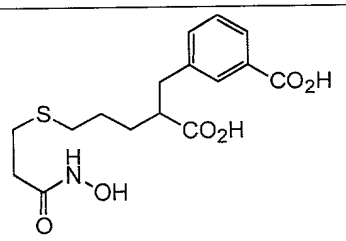
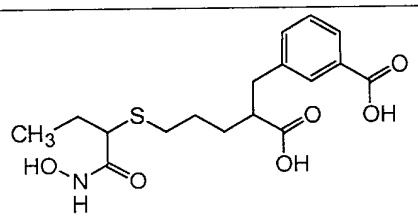
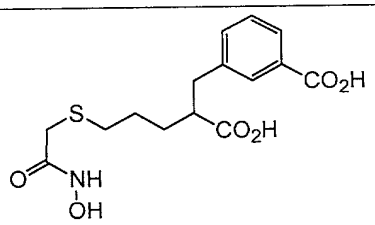
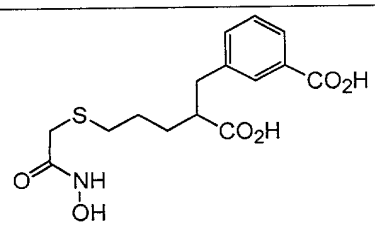
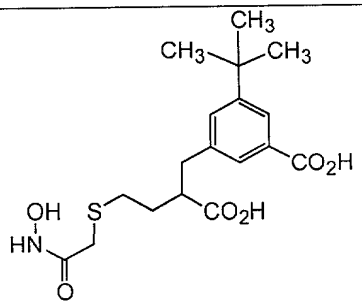
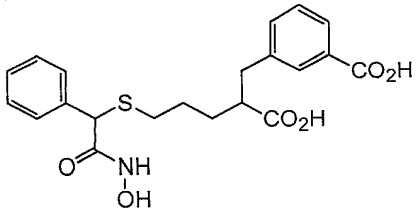
US20050250850A1

Structure	Name
	3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester
	3-(2-Carboxy-3-hydroxyamino-propyl)-benzoic acid
	3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester
	

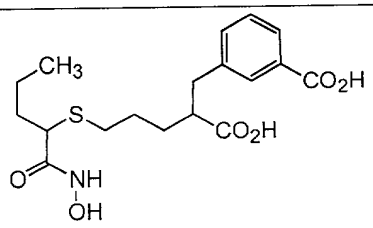
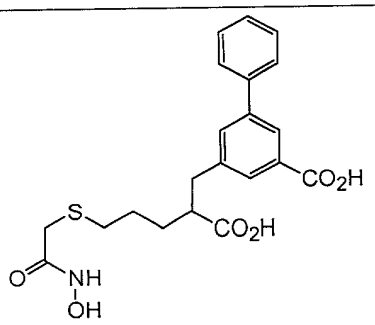
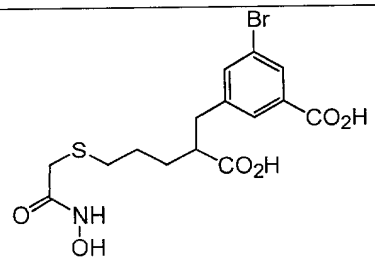
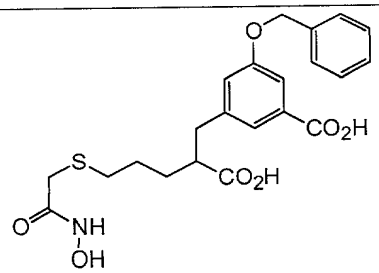
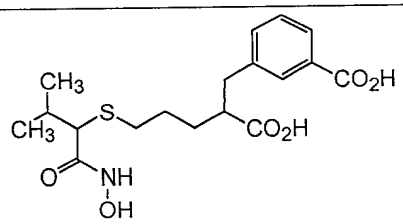
US 2005/0059705 A1

Structure	Name
	
	5-Hydroxycarbamoyl-isophthalic acid monoethyl ester
	6-Benzyloxy-N-hydroxy-isophthalamic acid methyl ester
	6,N-Dihydroxy-isophthalamic acid
	6-Benzyloxy-N-hydroxy-isophthalamic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid

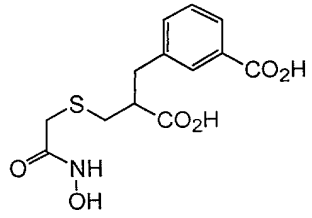
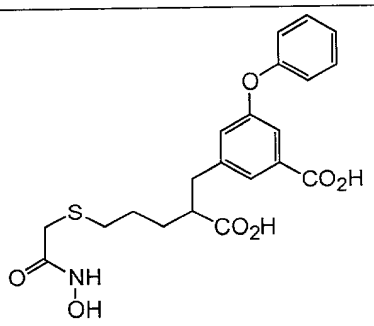
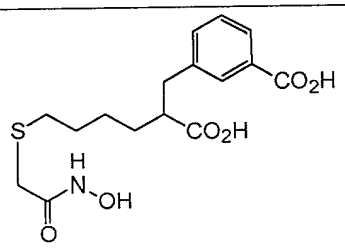
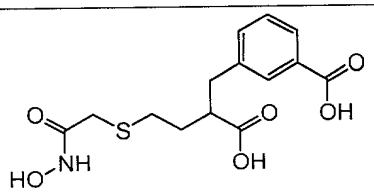
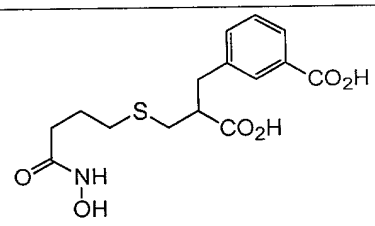
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Structure	Name
	3-[2-Carboxy-5-(2-hydroxycarbamoyl-ethylsulfanyl)-pentyl]-benzoic acid
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-propylsulfanyl)-pentyl]-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethylsulfanyl-pentyl)-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid
	3-[2-Carboxy-5-(hydroxycarbamoyl-phenyl-methylsulfanyl)-pentyl]-benzoic acid

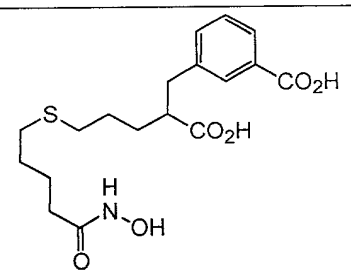
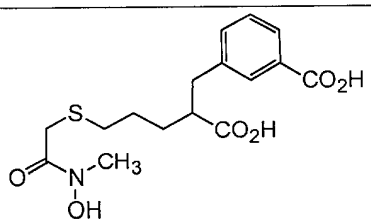
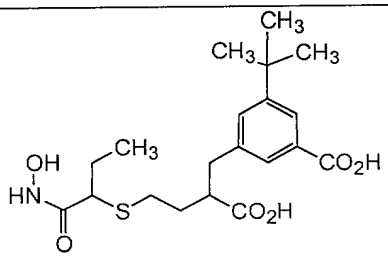
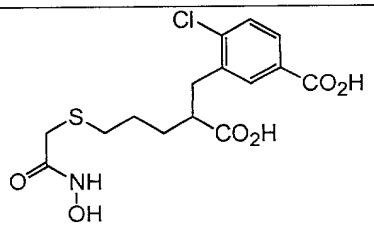
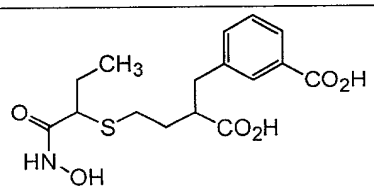
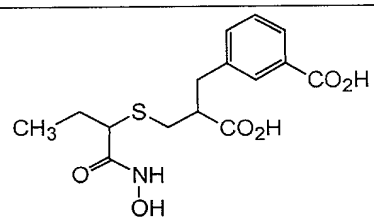
US20060004094A1

Structure	Name
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid
	5-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-biphenyl-3-carboxylic acid
	3-Bromo-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-Benzyloxy-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-2-methyl-propylsulfanyl)-pentyl]-benzoic acid

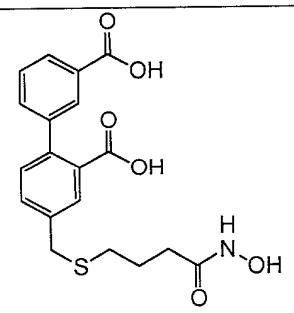
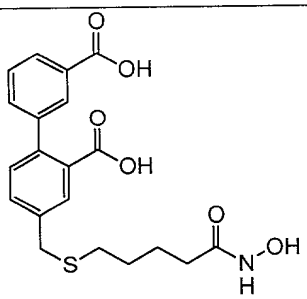
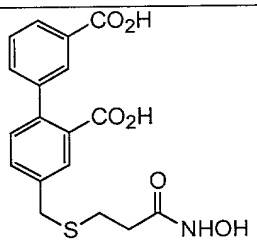
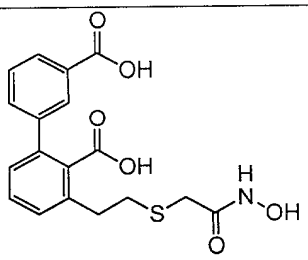
WO 2005/054201 PCT/US2004/040569

Structure	Name
	3-(2-Carboxy-3-hydroxycarbamoylmethyl-sulfanylpropyl)-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-5-phenoxy-benzoic acid
	3-(2-Carboxy-6-hydroxycarbamoylmethyl-sulfanylhexyl)-benzoic acid
	3-(2-Carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid
	3-[2-Carboxy-3-(3-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid

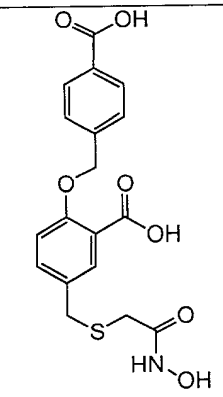
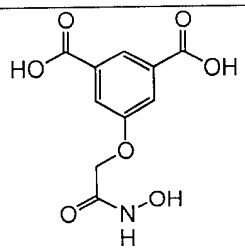
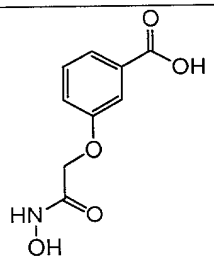
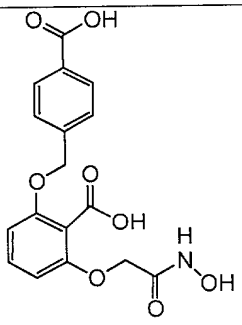
Patent Application Publication

Structure	Name
	3-[2-Carboxy-5-(4-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid
	3-{2-Carboxy-5-[(hydroxy-methyl-carbamoyl)-methylsulfanyl]-pentyl}-benzoic acid
	3-*tert*-Butyl-5-[2-carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-4-chloro-benzoic acid
	3-[2-Carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid
	3-[2-Carboxy-3-(1-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid

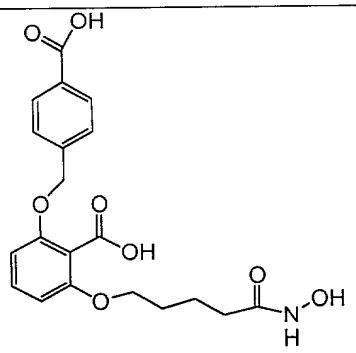
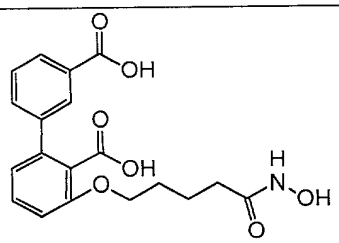
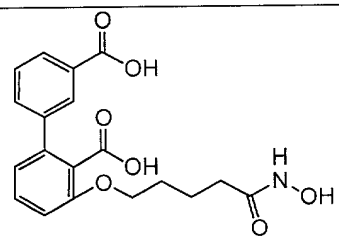
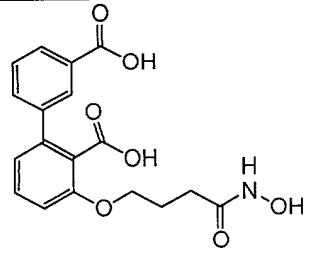
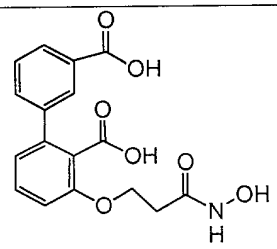
US 2005/0256202 A1

Structure	Name
	4-(3-Hydroxycarbamoyl-propylsulfanylmethyl)-biphenyl-2,3'-dicarboxylic acid
	4-(4-Hydroxycarbamoyl-butylsulfanylmethyl)-biphenyl-2,3'-dicarboxylic acid
	4-(2-Hydroxycarbamoyl-ethylsulfanylmethyl)-biphenyl-2,3'-dicarboxylic acid
	3-(2-Hydroxycarbamoyl-methylsulfanylethyl)-biphenyl-2,3'-dicarboxylic acid

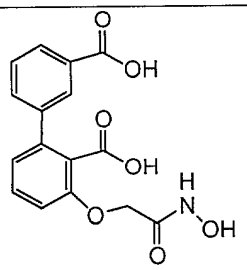
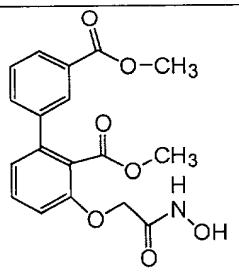
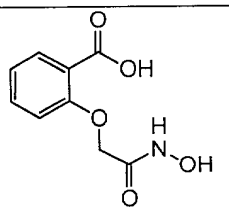
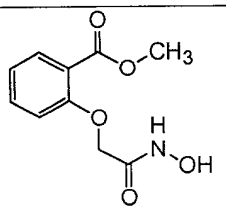
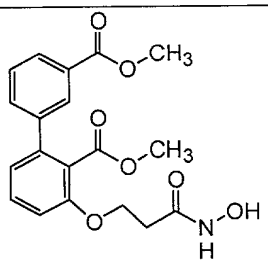
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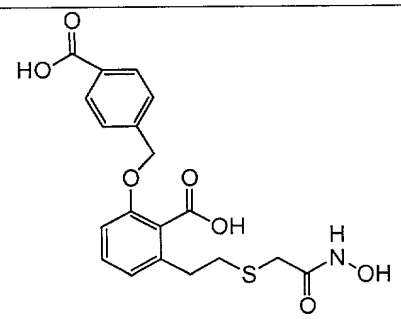
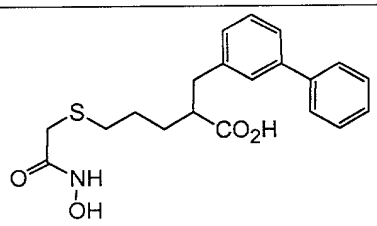
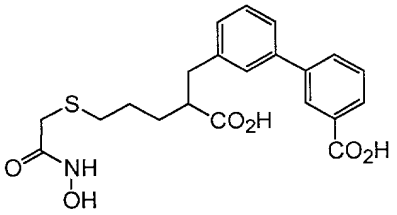
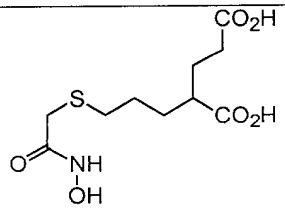
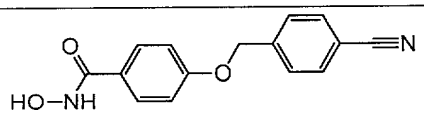
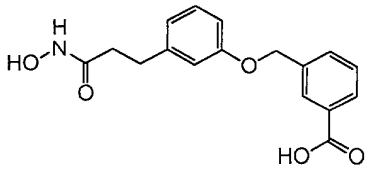
Structure	Name
	
	5-Hydroxycarbamoylmethoxy-isophthalic acid
	3-Hydroxycarbamoylmethoxy-benzoic acid
	

US 2005/0256128 A1

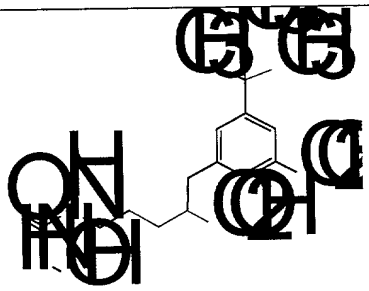
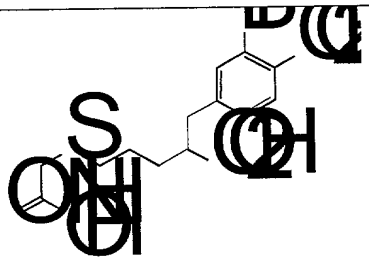
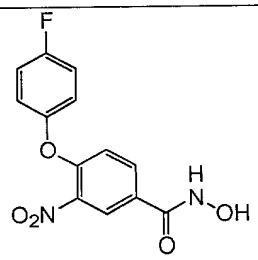
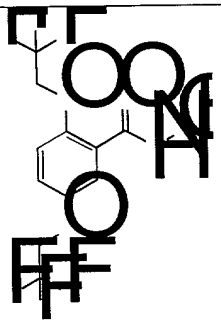
Structure	Name
	
	3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid
	3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid
	3-(3-Hydroxycarbamoyl-propoxy)-biphenyl-2,3'-dicarboxylic acid
	3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid

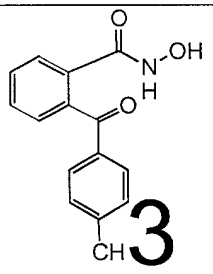
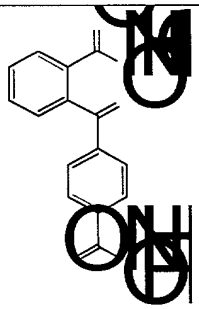
WO 2005/051890 PCT/US2004/039574

Structure	Name
	3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid
	3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid dimethyl ester
	2-Hydroxycarbamoylmethoxy-benzoic acid
	2-Hydroxycarbamoylmethoxy-benzoic acid methyl ester
	3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid dimethyl ester

Structure	Name
	
	2-Biphenyl-3-ylmethyl-5-hydroxycarbamoylmethylsulfanyl-pentanoic acid
	3'-(2-Carboxy-5-hydroxycarbamoylmethylsulfanyl-pentyl)-biphenyl-3-carboxylic acid
	2-(3-Hydroxycarbamoyl-methylsulfanylpropyl)-pentanedioic acid
	4-(4-Cyano-benzyloxy)-N-hydroxy-benzamide
	3-[3-(2-Hydroxycarbamoyl-ethyl)-phenoxymethyl]-benzoic acid

EP 1 503 769 B1

Structure	Name
	
	2-Bromo-4-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	2,N-Dihydroxy-benzamide
	4-(4-Fluoro-phenoxy)-N-hydroxy-3-nitro-benzamide
	N-Hydroxy-2,5-bis-(2,2,2-trifluoro-ethoxy)-benzamide

Structure	Name
	N-Hydroxy-2-(4-methyl-benzoyl)-benzamide
	

09655-0015P1

OTHER NAALADASE INHIBITORS

Other NAALADase inhibitors are described in International Publication No. WO 01/14390 and copending U.S. Patent Application No. 09/438,970 filed November 12, 1999 (corresponding to International Patent Application No. PCT/US00/30977 filed November 13, 2000), the entire contents of which publication and applications are herein incorporated by reference as though set forth herein in full.

Possible substituents of the compounds of formulas I-XV include, without limitation, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, hydroxy, carboxy, hydroperoxy, carbamido, carbamoyl, carbamyl, carbonyl, carbozoyl, amino,

hydroxyamino, formamido, formyl, guanyl, cyano, cyanoamino, isocyano, isocyanato, diazo, azido, hydrazino, triazano, nitrilo, nitro, nitroso, isonitroso, nitrosamino, imino, nitrosimino, oxo, C_1-C_6 alkylthio, sulfamino, sulfamoyl, sulfeno, sulfhydryl, sulfinyl, sulfo, sulfonyl, thiocarboxy, thiocyano, isothiocyano, thioformamido, halo, haloalkyl, chlorosyl, chloryl, perchloryl, trifluoromethyl, iodosyl, iodyl, phosphino, phosphinyl, phospho, phosphono, arsino, selanyl, disilanyl, siloxy, silyl, silylene and carbocyclic and heterocyclic moieties.

Carbocyclic moieties include alicyclic and aromatic structures. Examples of carbocyclic and heterocyclic moieties include, without limitation, phenyl, benzyl, naphthyl, indenyl, azulenyl, fluorenyl, anthracenyl, indolyl, isoindolyl, indolinyl, benzofuranyl, benzothiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, tetrahydrofuranyl, tetrahydropyranyl, pyridyl, pyrrolyl, pyrrolidinyl, pyridinyl, pyrimidinyl, purinyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, quinolizinyl, furyl, thiophenyl, imidazolyl, oxazolyl, benzoxazolyl, thiazolyl, isoxazolyl, isotriazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, trithianyl, indolizinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, thienyl, tetrahydroisoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, and

D-0005-PCT-US

phenoxazinyl.

All variables of formulas I-XV are independently selected at each occurrence. For example, formula II may have two different $CR^{10}R^{11}$ moieties when X is a moiety of formula III and n is 2, with the first $CR^{10}R^{11}$ moiety being CH_2, and the second $CR^{10}R^{11}$ moiety being $CH(CH_3)$.

The compounds of formulas I-XV may possess one or more asymmetric carbon center(s) and, thus, may be capable of existing in the form of optical isomers as well as in the form of racemic or non-racemic mixtures of optical isomers. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes well known in the art, for example by formation of diastereoisomeric salts by treatment with an optically active acid or base, and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. Examples of optically active acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid. A different process for separation of optical isomers involves the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules, for example, esters, amides, acetals, ketals, and the like, by reacting compounds used in the inventive methods and pharmaceutical compositions with an optically active acid in an activated form, an

optically active diol or an optically active isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then hydrolyzed to deliver the enantiomerically pure compound. In some cases hydrolysis to the parent optically active drug is not necessary prior to dosing the patient since the compound can behave as a prodrug. The optically active compounds can likewise be obtained by utilizing optically active starting materials.

It is understood that the compounds of formulas I-XV encompass optical isomers as well as racemic and non-racemic mixtures.

SYNTHESIS OF NAALADASE INHIBITORS

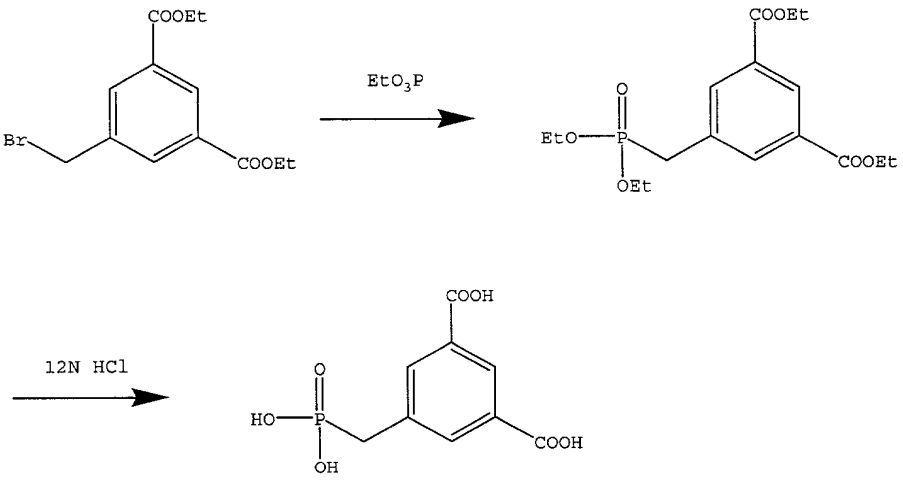
Some of the NAALADase inhibitors used in the inventive methods and pharmaceutical compositions can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways and examples depicted in U.S. Patents Nos. 5,672,592, 5,795,877, 5,863,536, 5,880,112, 5,902,817, 5,962,521, 5,968,915, 6,025,344, 6,025,345, 6,028,216, 6,046,180, 6,054,444, 6,071,965 and 6,121,252, allowed U.S. Patent Application No. 09/228,391 for which the issue fee has been paid, copending U.S. Patent Application No. 09/438,970 filed November 12, 1999 (corresponding to International Patent Application No. PCT/US00/30977 filed November 13, 2000), and International Publications Nos. WO

99/33849, WO 00/01668 and WO 01/14390, the entire contents of which patents, patent application and publications are herein incorporated by reference, as though set forth herein in full.

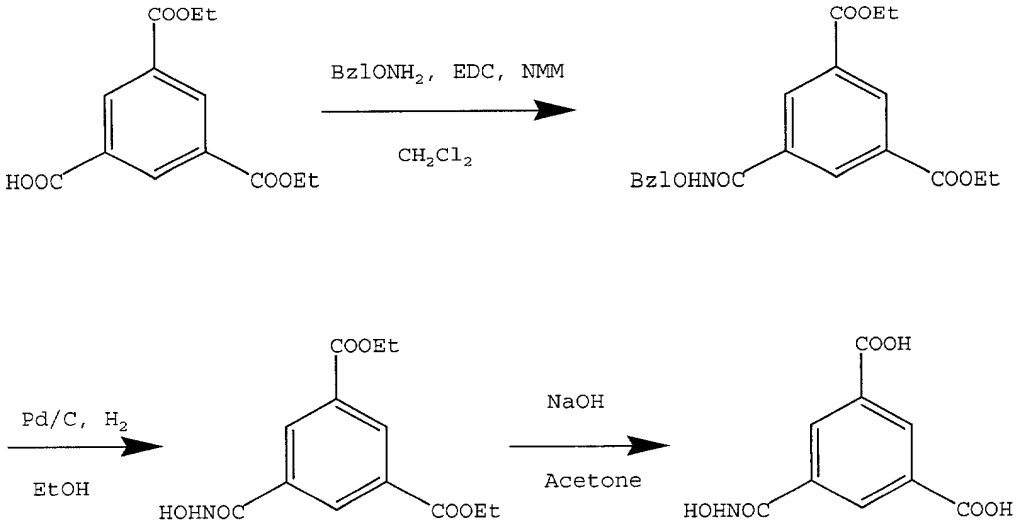
Other NAALADase inhibitors may be available from commercial suppliers or can be readily prepared by an ordinarily skilled artisan using standard techniques such as those disclosed in U.S. Patent No. 5,859,046, the entire contents of which reference are herein incorporated by reference as though set forth herein in full.

Yet other NAALADase inhibitors can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below in SCHEMES I-XVI.

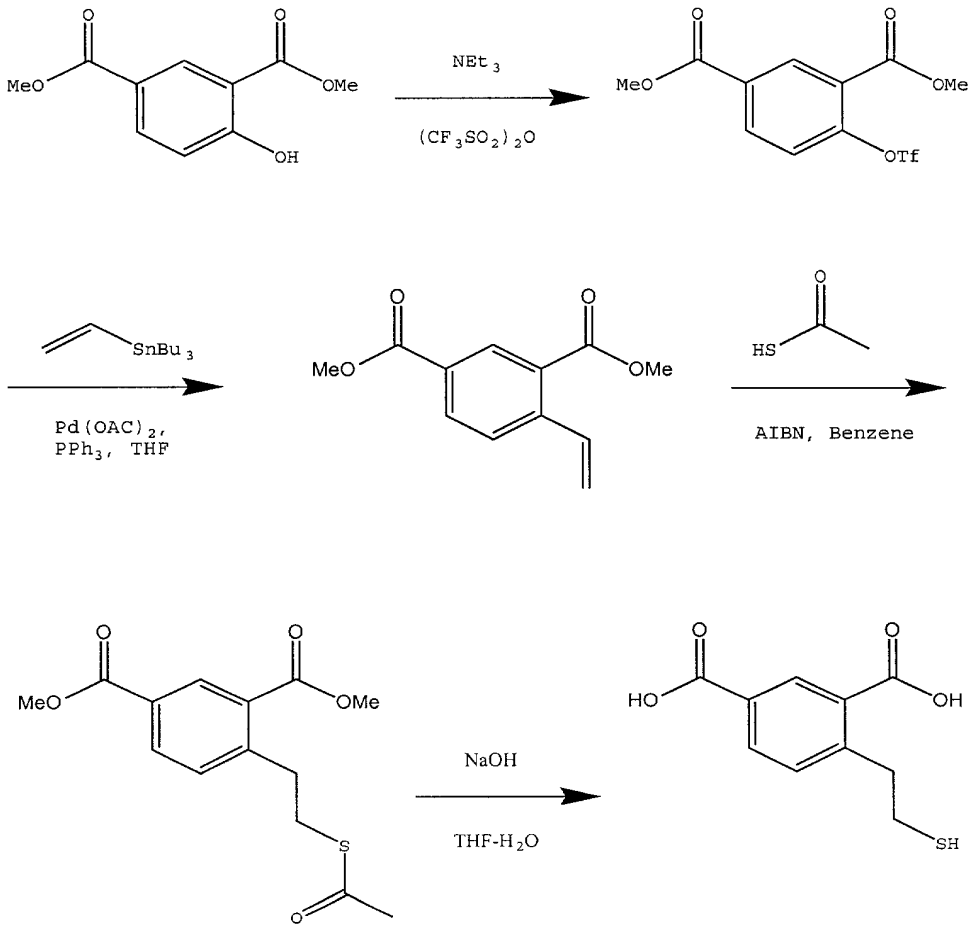
SCHEME I



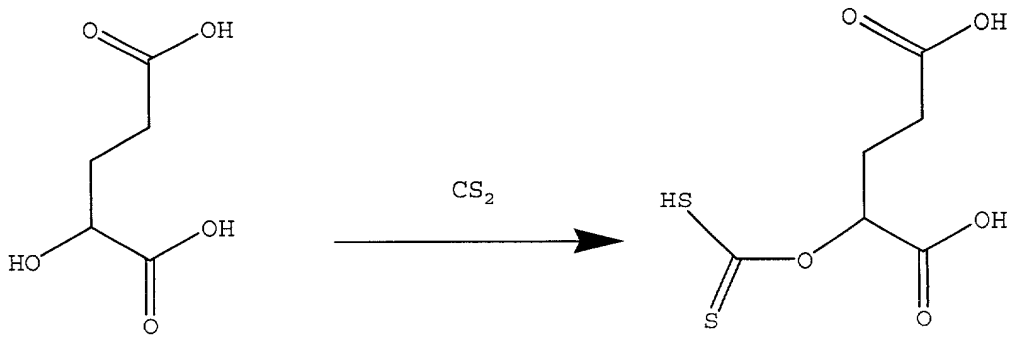
SCHEME II



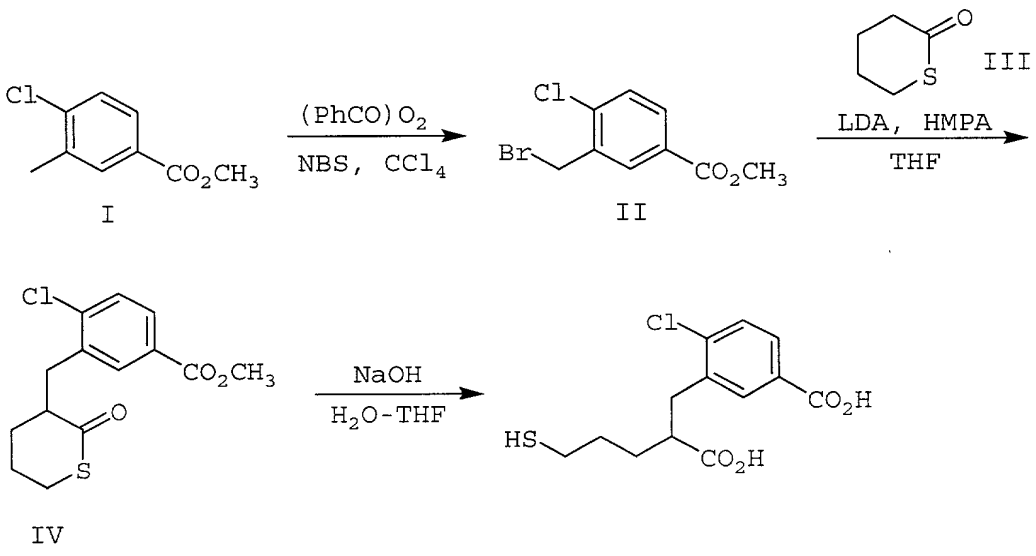
SCHEME III


MeO
OMe
OH
NEt3
(CF3SO2)2O
OTf
SnBu3
Pd(OAC)2,
PPh3, THF
HS
AIBN, Benzene
NaOH
THF-H2O
HO
SH

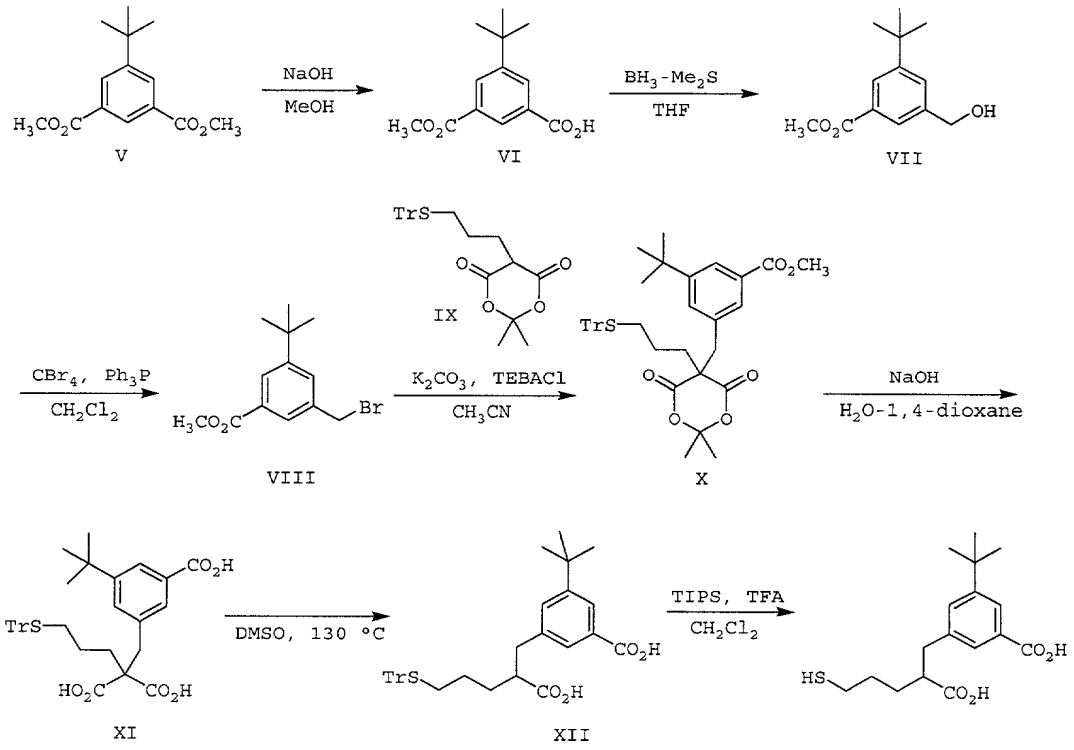
SCHEME IV



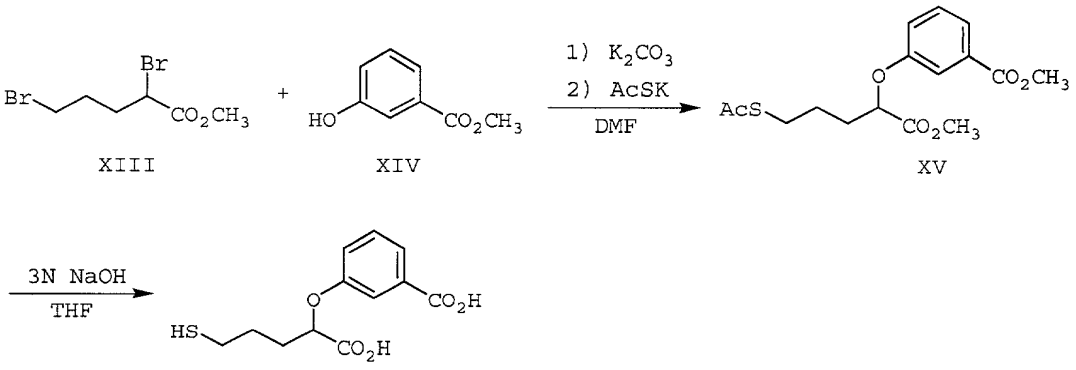
SCHEME V



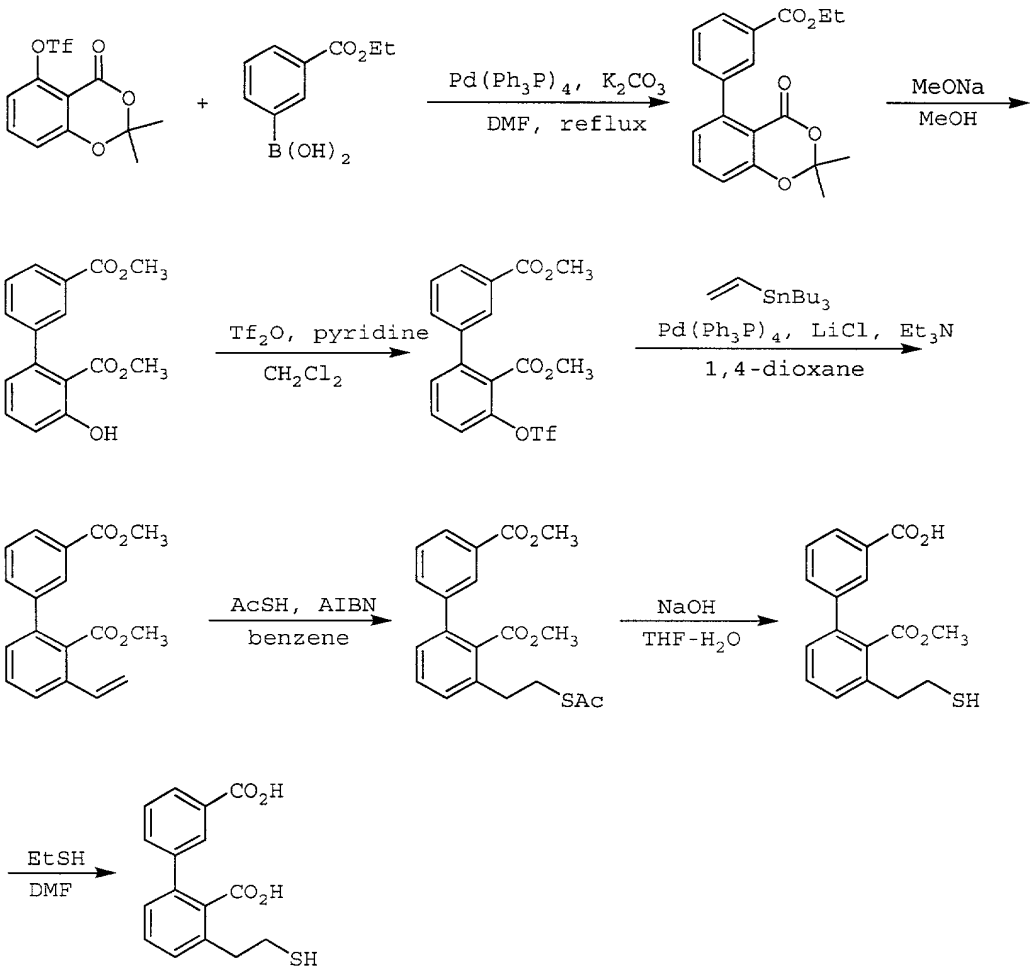
SCHEME VI



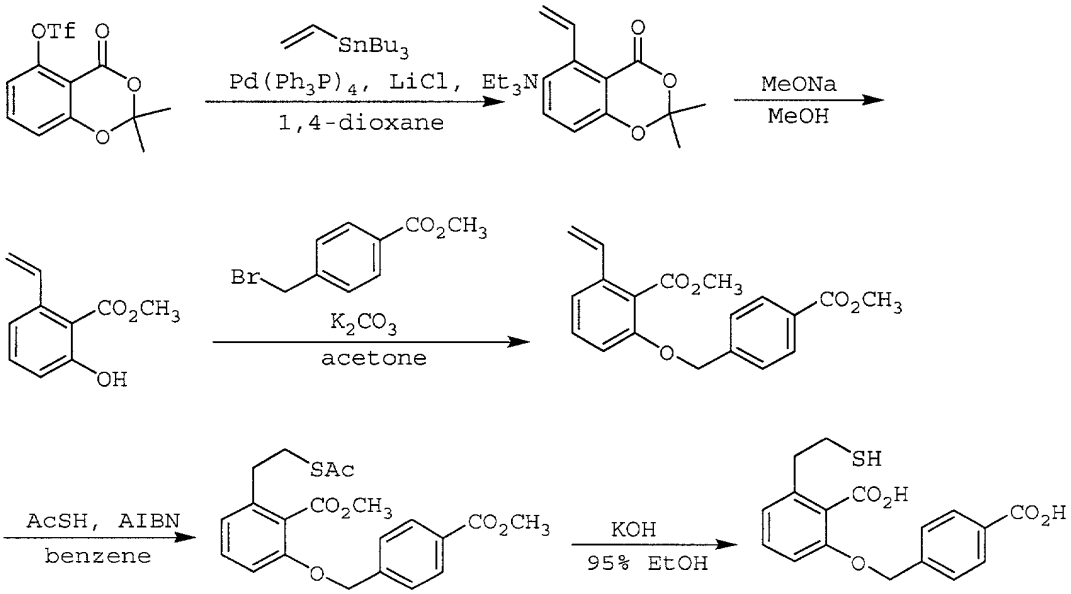
SCHEME VII



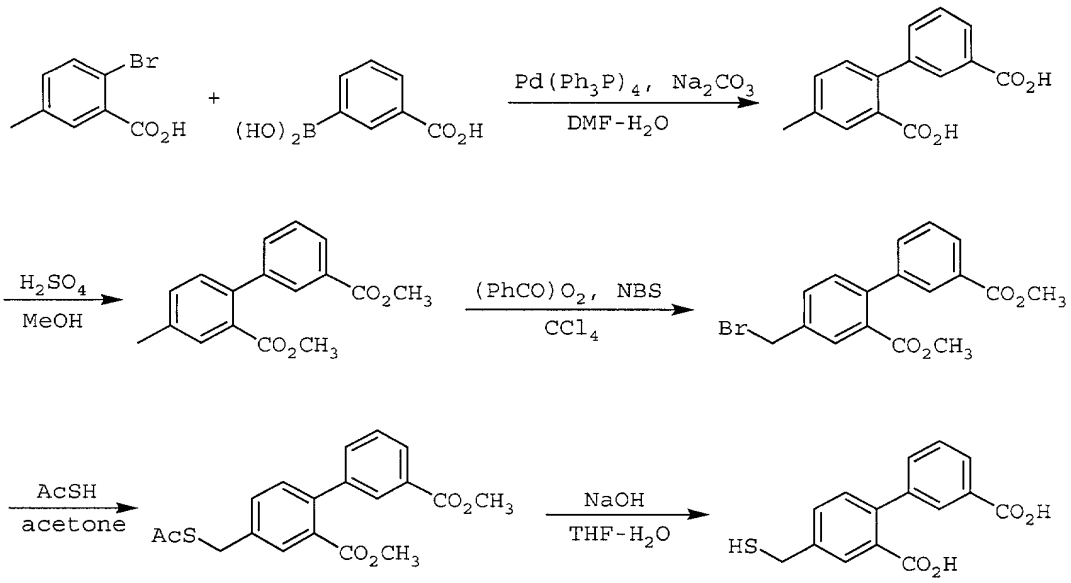
SCHEME VIII


OTf
O
O
O
+
CO2Et
B(OH)2
Pd(Ph3P)4, K2CO3
DMF, reflux
CO2Et
O
O
O
MeONa
MeOH
CO2CH3
CO2CH3
OH
Tf2O, pyridine
CH2Cl2
CO2CH3
CO2CH3
OTf
SnBu3
Pd(Ph3P)4, LiCl, Et3N
1,4-dioxane
CO2CH3
CO2CH3
AcSH, AIBN
benzene
CO2CH3
CO2CH3
SAc
NaOH
THF-H2O
CO2H
CO2CH3
SH
EtSH
DMF
CO2H
CO2H
SH

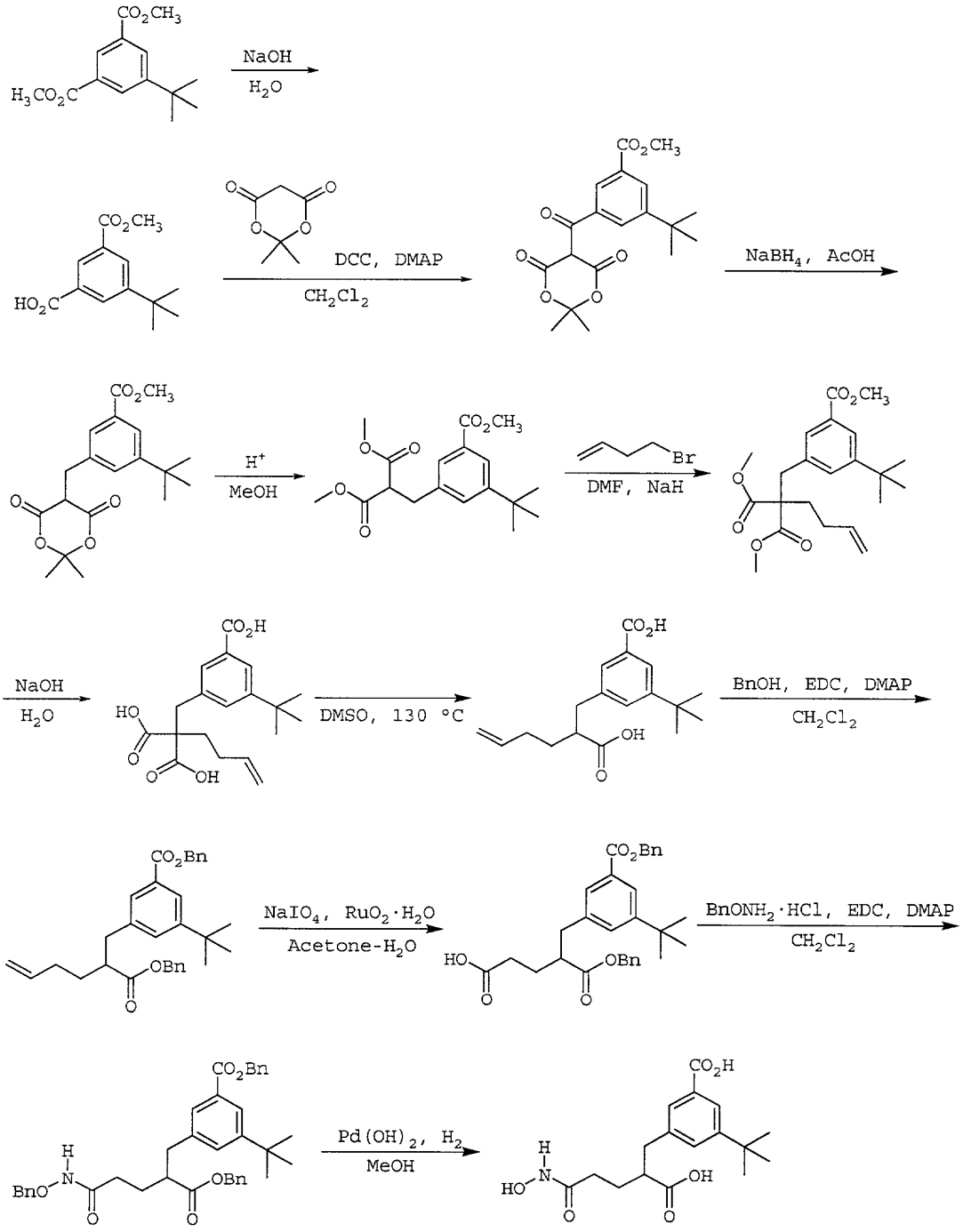
SCHEME IX



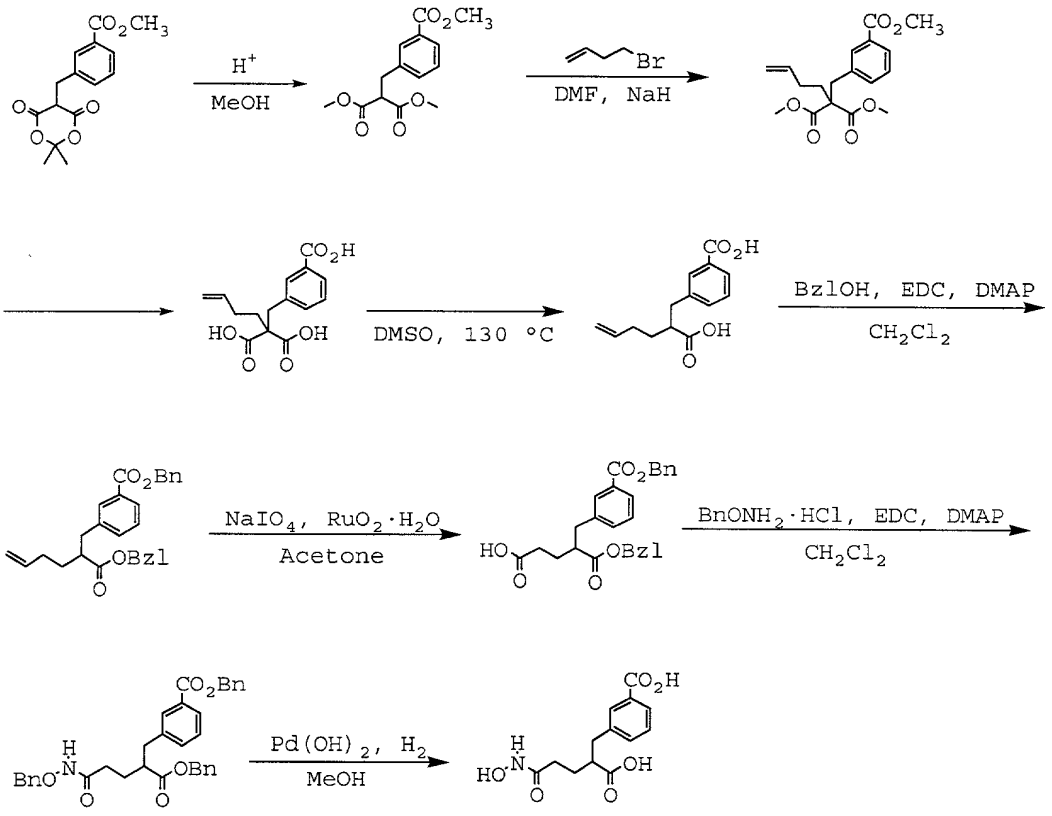
SCHEME X



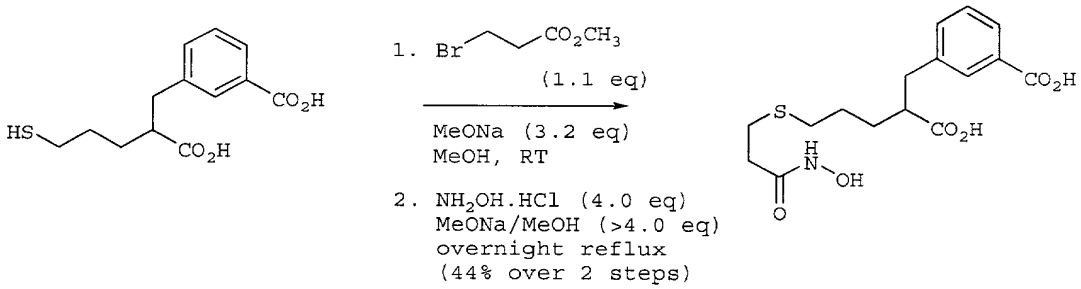
SCHEME XI


NaOH
H2O
DCC, DMAP
CH2Cl2
NaBH4, AcOH
H+
MeOH
DMF, NaH
NaOH
H2O
DMSO, 130 °C
BnOH, EDC, DMAP
CH2Cl2
NaIO4, RuO2·H2O
Acetone-H2O
BnONH2·HCl, EDC, DMAP
CH2Cl2
Pd(OH)2, H2
MeOH

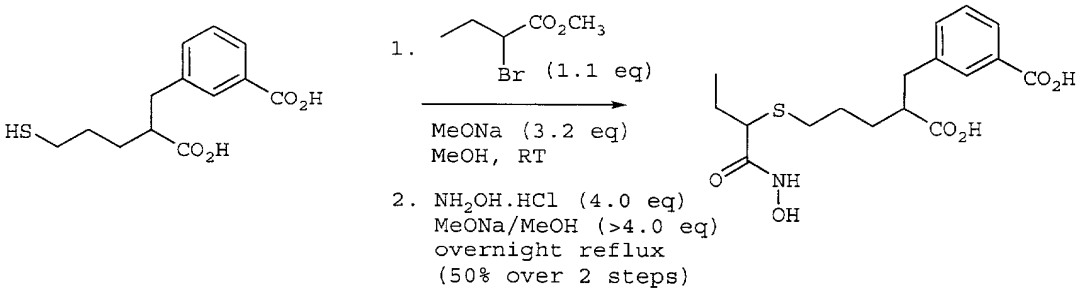
SCHEME XII



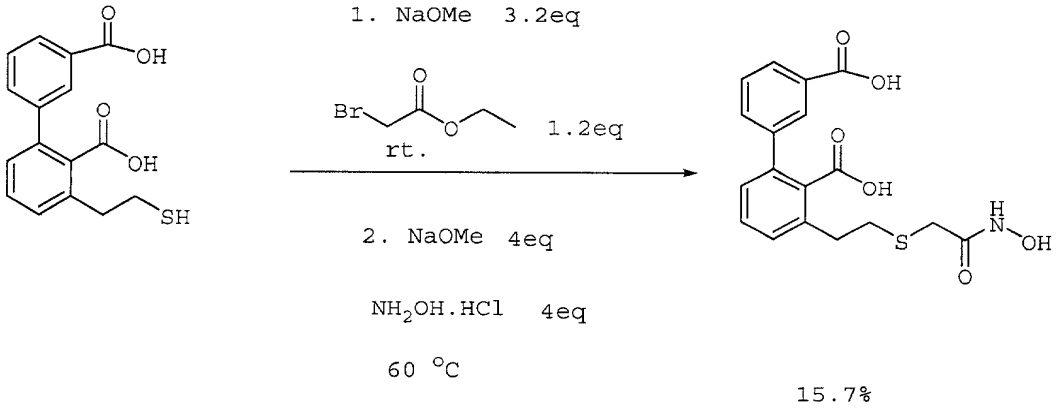
SCHEME XIII



SCHEME XIV



SCHEME XV

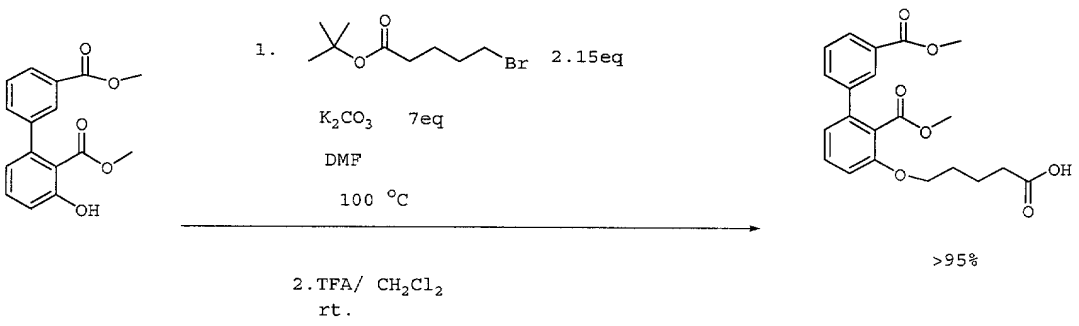


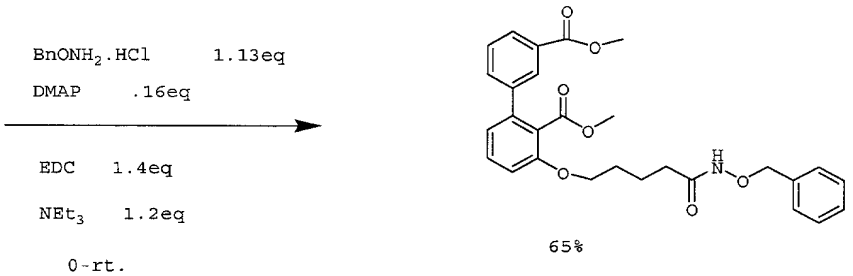
15.7%

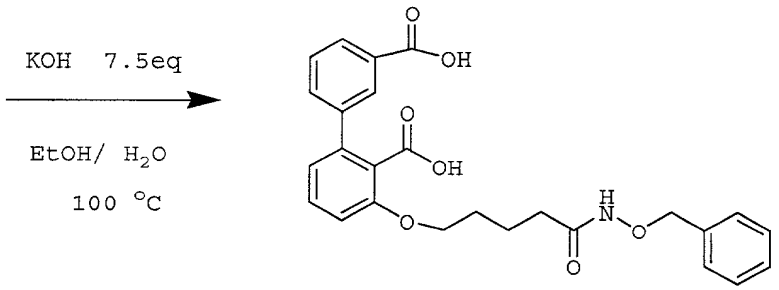
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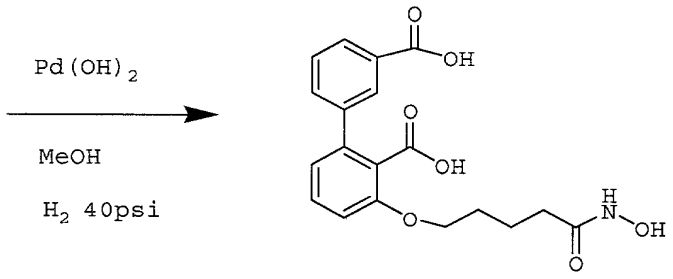
5

SCHEME XVI


1.
2.15eq
K_2CO_3 7eq
DMF
100 °C
2.TFA/ CH_2Cl_2
rt.
OH
>95%

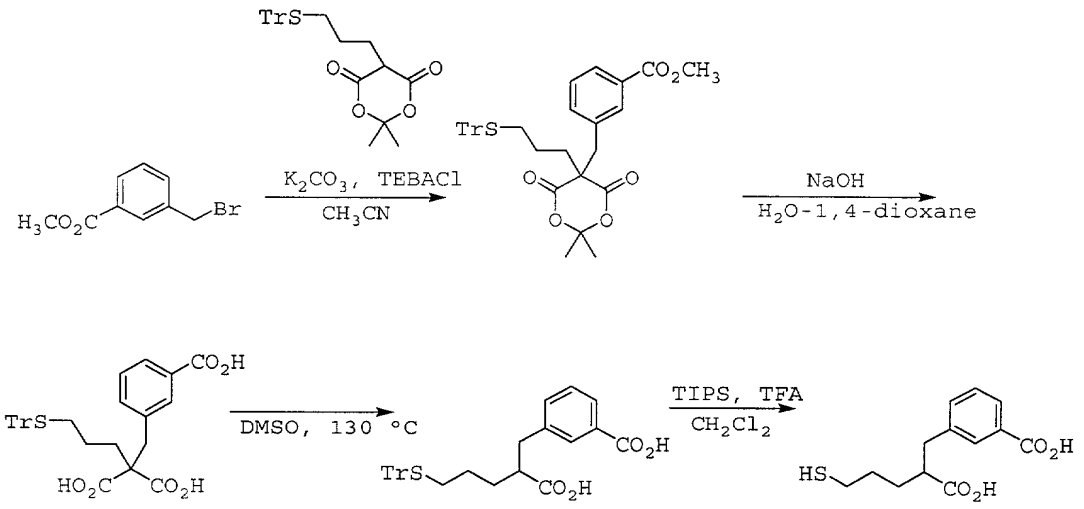

$BnONH_2.HCl$ 1.13eq
DMAP .16eq
EDC 1.4eq
NEt_3 1.2eq
0-rt.
65%


KOH 7.5eq
EtOH/ H_2O
100 °C
OH
62%

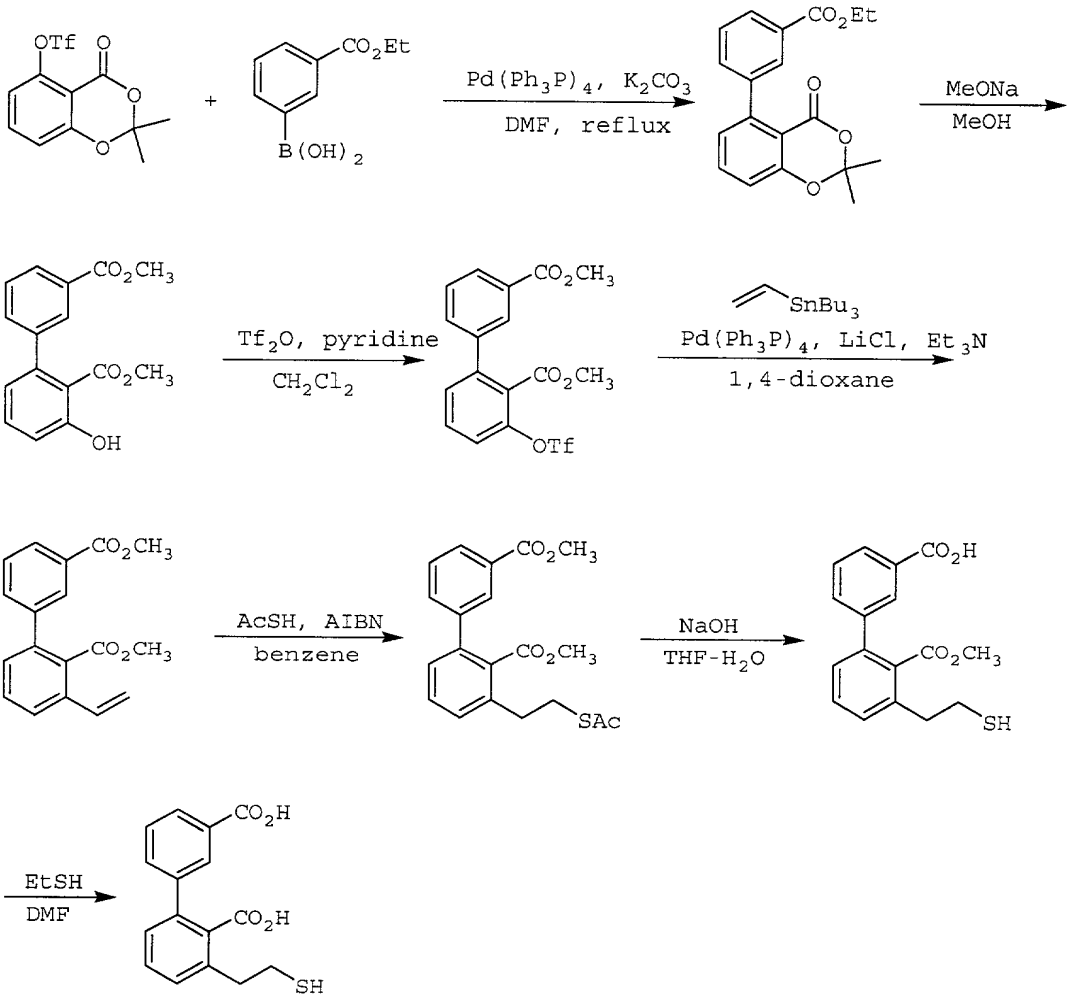

$Pd(OH)_2$
MeOH
H_2 40psi
OH
83%

Precursor compounds may be commercially available, prepared by methods known to a person of skill in the art, or prepared by SCHEMES XVII and XVIII.

SCHEME XVII



SCHEME XVIII



ROUTE OF ADMINISTRATION

In the inventive methods, the compounds will generally be administered to a patient in the form of a pharmaceutical formulation. Such formulation preferably includes, in addition to the active agent, a physiologically acceptable carrier and/or diluent. The compounds may be administered locally or systemically by any means known to an ordinarily skilled artisan. For example, the compounds may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, intracranial or intraosseous injection and infusion techniques. Topical administration includes, without limitation, administration via eyedrops. The exact administration protocol will vary depending upon various factors including the age, body weight, general health, sex and diet of the patient; the determination of specific administration procedures would be routine to an ordinarily skilled artisan. The compounds and compositions used in the inventive methods may be capable of crossing the blood-brain barrier.

Docket No. 5050-P-US-CON2

DOSAGE

In the inventive methods, the compounds and compositions may be administered by a single dose, multiple discrete doses or continuous infusion. Pump means, particularly subcutaneous pump means, are preferred for continuous infusion.

Dose levels on the order of about 0.001 to about 10,000 mg/kg of the active ingredient compound are useful in the inventive methods, with preferred levels being about 0.1 to about 1,000 mg/kg, and more preferred levels being about 1 to 100 mg/kg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity and the possible toxicity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being treated; and the form of administration. Typically, *in vitro* dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models are also helpful. The considerations for determining the proper dose levels are well known in the art.

ADMINISTRATION REGIMEN

For the inventive methods, any administration regimen well known to an ordinarily skilled artisan for regulating the timing and sequence of drug delivery can be used and

repeated as necessary to effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

CO-ADMINISTRATION WITH OTHER TREATMENTS

In the inventive methods, the NAALADase inhibitors and pharmaceutical compositions may be used alone or in combination with one or more additional agent(s) for simultaneous, separate or sequential use.

The additional agent(s) may be any therapeutic agent(s) known to an ordinarily skilled artisan, including, without limitation, (an)other compound(s) of formulas I-XV.

The NAALADase inhibitors and pharmaceutical compositions may be co-administered with one or more therapeutic agent(s) either (i) together in a single formulation, or (ii) separately in individual formulations designed for optimal release rates of their respective active agent. Each formulation may contain from about 0.01% to about 99.99% by weight of a NAALADase inhibitor, as well as one or more pharmaceutically acceptable carrier(s), such as wetting, emulsifying and/or pH buffering agent(s).

In addition, the NAALADase inhibitors and pharmaceutical compositions may be administered prior to, during or following surgery or physical therapy.

PCT/US95/12936

EXAMPLES

The following examples are illustrative of the present invention and are not intended to be limitations thereon. Unless otherwise indicated, all percentages are based upon 100% by weight of the final composition.

EXAMPLE 1

Preparation of 5-phosphonomethyl-1,3-benzenedicarboxylic acid (SCHEME I)

Diethyl 5-[(diethoxyphosphinyl)methyl]-1,3-benzenedicarboxylate

A solution of 5-bromomethyl-1,3-benzene-dicarboxylate (Collman et al., *J. Am. Chem. Soc.*, 116(14) (1994) 6245-6251; 0.315 g, 1.0 mmol) in triethylphosphite (3.0 mL) was heated at 150° C for 5 hours. The solvent was removed under reduced pressure and the residual oil was purified by chromatography to give 0.248 g of colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H), 1.42 (t, 3H), 3.26 (d, 2H), 4.06 (q, 2H), 4.41 (q, 2H), 8.17 (s, 2H), 8.58 (s, 1H). TLC: R_f 0.10 (EtOAc/Hexanes 1/1).

5-Phosphonomethyl-1,3-benzenedicarboxylic acid

A solution of diethyl 5-[(diethoxyphosphinyl) methyl]-1,3-benzenedicarboxylate (0.186 g, 0.5 mmol) in 12 N HCl (2.5 mL) was heated at 100° C for 24 hours. The resulting precipitate was washed with water and dried under vacuum to give 0.057 g of white powder: ^{1}H NMR (D_2O) δ 3.11 (d, 2H), 7.93 (s, 2H), 8.19 (s, 1H). TLC: R_f 0.20

(EtOAc/Hexanes 1/1). Elemental analysis calculated for $C_9H_7O_7P \cdot H_2O$: C, 38.86; H, 3.99. Found: C, 38.74; H, 4.08.

EXAMPLE 2

Preparation of 5-[(hydroxyamino)carbonyl]-1,3-benzene-dicarboxylic acid (SCHEME II)

Diethyl 5-[[(phenylmethoxy)amino]carbonyl]-1,3-benzenedicarboxylate

To a solution of diethyl 1,3,5-benzenetricarboxylate (3.192 g, 20 mol) and O-benzylhydroxyamine hydrochloride (4.789 g, 19 mmol) in 40 mL were added N-methylmorpholine (2.2 mL, 20 mmol) and EDC (3.834 g, 20 mmol) at 0° C, and the mixture was stirred at room temperature for 20 hours. The solvent was removed by evaporator and the residue was dissolved in EtOAc (150 mL). The organic solution was washed with 1 N HCL (150 mL), washed with saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, and concentrated to give white solid. This material was recrystallized from EtOAc to give 4.154 g of white powder: ^1H NMR ($CDCl_3$) δ 1.41 (t, 6H), 4.40 (q, 4H), 5.05 (s, 2H), 7.3-7.5 (m, 5H), 8.52 (s, 2H), 8.76 (s, 1H), 9.1 (br, 1H). TLC: R_f 0.62 (EtOAc/Hexanes 1/1).

Diethyl 5-[(hydroxyamino)carbonyl]-1,3-benzenedicarboxylate

To a solution of diethyl 5-[[(phenylmethoxy)amino]carbonyl]-1,3-benzenedicarboxylate (0.742 g, 2.0 mmol) in ethanol (10 mL) was added a

suspension of Pd/C in ethanol (5 mL), and the mixture was shaken under hydrogen (50 psi) for 20 hours. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated to give white powder. This material was washed with ethanol (10 mL x 2) and dried under vacuum to give 0.380 g of white powder: ^{1}H NMR (CD_3OD) δ 1.44 (t, 6H), 4.45 (q, 4H), 8.60 (s, 2H), 8.72 (s, 1H). TLC: R_f 0.20 (EtOAc/Hexanes 1/1).

5-[(Hydroxyamino)carbonyl]-1,3-benzene-dicarboxylic acid

To a solution of diethyl 5-[(hydroxyamino)carbonyl]-1,3-benzenedicarboxylate (0.281 g, 1.0 mmol) in acetone (5 mL) was added 1.0 N NaOH (5 mL) at room temperature, and the mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was taken up with 1 N HCl (15 mL) to give white precipitate. This material was dried under vacuum to give 0.096 g of white solid: ^{1}H NMR (D_2O) δ 8.52 (s, 2H), 8.76 (s, 1H). Elemental analysis calculated for $C_9H_7NO_6 \cdot H_2O$: C, 44.45; H, 3.73; N, 5.76. Found: C, 44.47; H, 3.78; N, 5.74.

EXAMPLE 3

Preparation of 4-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid (SCHEME III)

Dimethyl 4-trifluoromethanesulfonyloxy-1,3-benzenedicarboxylate

To a solution of dimethyl 4-hydroxy-isophthalate (0.850 g, 4.04 mmol) in CH_2Cl_2 (15 mL) were added triethylamine (0.6 mL, 4.3 mmol) and triflic anhydride (0.8 mL, 4.76 mmol) at 0° C, and the mixture was stirred at 0° C for 18 hours. The solvent was evaporated and the residue was diluted with ether (30 mL). The organic solution was washed with 1 N HCl (30 mL x 3), dried over $MgSO_4$, and concentrated to give 1.30 g of dark yellow oil (93% yield): ^1H NMR ($CDCl_3$) δ 3.97 (s, 3H), 4.00 (s, 3H), 7.4 (d, 1H), 8.3 (d, 1H), 8.74 (s, 1H).

Dimethyl 4-ethenyl-1,3-benzenedicarboxylate

To a solution of dimethyl 4-trifluoromethanesulfonyl-oxy-1,3-benzenedicarboxylate (1.5 g, 4.38 mmol) in dioxane (50 mL) were added $Pd(PPh_3)_4$ (510 mg, 0.44 mmol), lithium chloride (1.3 g, 30.7 mmol) and tributyl(vinyl)tin (1.5 mL, 5.13 mmol) at room temperature. The mixture was heated at 100° C for 5 hours. The reaction mixture was filtered and the filtrate was concentrated and passed through a column of silica gel (Hexanes/EtOAc = 10:1) to give 1.1 g of colorless oil (84% yield): ^1H NMR: ($CDCL_3$) δ 3.92 (s, 3H), 3.93 (s, 3H), 5.45 (d, 1H), 5.73 (d, 1H), 7.49 (m, 1H), 7.66 (d, 1H), 8.13 (d, 1H), 8.53 (s, 1H).

Dimethyl 4-[2-(acetylthio)ethyl]-1,3-benzenedicarboxylate

To a degassed solution of dimethyl 4-ethenyl-1,3-benzenedicarboxylate (415 mg, 1.88 mmol) in benzene (6 mL) were added AIBN (33 mg, 0.21 mmol) and thioacetic acid (0.27 mL, 3.78 mmol), and the mixture was refluxed for 5

hours. The reaction mixture was diluted with aqueous $NaHCO_3$ solution (15 mL) and extracted with EtOAc (15 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residual material was purified by silica gel chromatography (hexanes/EtOAc = 10:1) to give 0.150 g of colorless oil (27% yield): ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H), 3.16 (t, 2H), 3.28 (t, 2H), 3.94 (s, 6H), 7.42 (d, 1H), 8.09 (d, 1H), 8.58 (s, 1H).

4-(2-Mercaptoethyl)-1,3-benzenedicarboxylic acid

To a degassed solution of dimethyl 4-[2-(acetylthio)ethyl]-1,3-benzenedicarboxylate (0.130 g, 0.44 mmol) in THF (5 mL) was added a degassed solution of 5 N NaOH (5 mL). The reaction mixture was stirred under nitrogen overnight. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAC (10 mL). The organic layer was dried over $MgSO_4$ and concentrated to give 0.045 g of white solid (45% yield): ^1H NMR (DMSO) δ 2.67 (t, 2H), 3.21 (t, 2H), 7.37 (d, 1H), 7.98 (d, 1H), 8.46 (s, 1H). ^{13}C NMR (DMSO) δ 26.64, 40.60, 130.87, 132.05, 133.46, 133.81, 134.13, 148.53, 169.22, 170.20. Elemental analysis calculated for $C_{10}H_{10}SO_4$: C, 53.09; H, 4.45; S, 14.47. Found: C, 53.37; H, 4.87; S, 12.84. MS(FAB): 225.

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EXAMPLE 4

Preparation of 5-carboxy-2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid (Scheme V)

Methyl 3-bromomethyl-4-chlorobenzoate **II**

To a suspension of methyl 4-chloro-3-methylbenzoate **I** (19.9 g, 108 mmol) and *N*-bromosuccinimide (NBS, 20.2 g, 114 mmol) in carbon tetrachloride (500 mL) was added benzoyl peroxide (1.30 g, 5.4 mmol), and the mixture was stirred at 90 °C overnight. The mixture was then cooled and the white precipitate was removed by filtration. The filtrate was concentrated and the resulting solid was re-crystallized from ethyl acetate to give methyl 3-bromomethy-4-chlorobenzoate **II** (15.0 g, 57 mmol, 53%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 4.63 (s, 2H), 7.49 (d, J = 8.3 Hz, 1H), 7.94 (dd, J = 2.1, 8.3 Hz, 1H), 8.15 (d, J = 2.1 Hz, 1H).

3-(2-Chloro-5-methoxycarbonylbenzyl)-tetrahydrothiopyrane-2-one **IV**

To a solution of lithium diisopropylamide (2.0 M solution, 3.3 mL, 6.6 mmol) in THF (25 mL) was added tetrahydrothiopyran-2-one **III** (0.731 g, 6.3 mmol) at -40 °C, and the mixture was stirred at -40 °C for 45 minutes. A solution of methyl 3-bromomethy-4-chlorobenzoate **II** (1.67 g, 6.3 mmol) in THF (10 mL) was then dropwise added to the mixture at -40 °C. Subsequently, hexamethylphosphoramide (0.20 g, 1.4 mmol) was added to

the mixture at -40 °C, and the reaction mixture was stirred at -40 °C for 4 hours. A saturated ammonium chloride solution (30 mL) was added to the reaction mixture, and the organic solvent was removed under reduced pressure. The mixture was then partitioned between ether (150 mL) and H_2O (150 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude material was chromatographed on silica gel using EtOAc/hexanes to afford 3-(2-chloro-5-methoxycarbonylbenzyl)-tetrahydrothio-pyrane-2-one **IV** (0.60 g, 2.0 mmol, 32%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.65-1.75 (m, 1H), 1.90-2.05 (m, 2H), 2.05-2.15 (m, 2H), 2.74 (dd, J = 9.4, 13.9 Hz, 1H), 2.85-3.00 (m, 1H), 3.10-3.20 (m, 2H), 3.58 (dd, J = 4.7, 13.9 Hz, 1H), 3.92 (s, 3H), 7.44 (d, J = 8.3 Hz, 1H), 7.85 (dd, J = 8.3, 2.1 Hz, 1H), 7.91 (d, J = 2.1 Hz, 1H).

5-Carboxy-2-chloro-alpha-(3-mercaptopropyl) benzenepropanoic acid

A solution of 3-(2-Chloro-5-methoxycarbonyl-benzyl)tetrahydrothiopyrane-2-one **IV** (9.26 g, 31.0 mmol) in THF (70 mL) was purged for 15 minutes with nitrogen. A degassed aqueous sodium hydroxide solution (2.2 M, 70 mL, 154 mmol) was added to the solution and the mixture was stirred at room temperature under nitrogen overnight. The reaction mixture was washed with ether, acidified by 3N HCl at 0 °C, and extracted with ether. The extract was

dried over $MgSO_4$ and concentrated to afford 5-carboxy-2-chloro-alpha-(3-mercaptopropyl) benzenepropanoic acid (8.42 g, 27.8 mmol, 90%) as a white solid: ^1H NMR (CD_3OD) δ 1.50-1.80 (m, 4H), 2.35-2.50 (m, 2H), 2.65-2.75 (m, 1H), 2.91 (dd, *J* = 6.2, 13.8 Hz, 1H), 2.96 (dd, *J* = 8.8, 13.8 Hz, 1 H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.76 (dd, *J* = 2.0, 8.3 Hz, 1H), 7.86 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (CD_3OD) δ25.1, 32.5, 33.2, 37.4, 46.8, 130.8, 131.2, 134.0, 139.1, 140.5, 169.2, 178.9. Elemental analysis calculated for $C_{13}H_{15}ClO_4S$: C, 51.57; H, 4.99; S, 10.59; Cl, 11.71. Found: C, 51.59; H, 4.94; S, 10.43; Cl, 11.80.

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EXAMPLE 5

Preparation of 3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid (Scheme VI)

Methyl 5-*tert*-butylhydrogenisophthalate **VI**

To a solution of dimethyl 5-*tert*-butylisophthalate **V** (23.0 g, 92 mmol) in methanol (150 mL) was added a solution of sodium hydroxide (3.68 g, 92 mmol) in H_2O (10 mL) at 25 °C, and the mixture was stirred at 25 °C for 3 hours. The organic solvent was removed under reduced pressure and the residual solid was suspended in an aqueous sulfuric acid solution (1.0 M). The suspension was filtered and the precipitate was washed with H_2O, dried under vacuum, and crystallized from hexanes/ethyl acetate to afford methyl 5-*tert*-butylhydrogenisophthalate **VI** (16.3

g, 69.0 mmol, 75%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.9 (s, 3H), 8.5 (s, 1H), 8.7 (s, 1H), 8.8 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 31.3 (3C), 35.2, 52.5, 128.8, 129.7, 130.7, 131.1, 131.6, 132.0, 166.7, 171.5.

Methyl 3-*tert*-butyl-5-hydroxymethylbenzoate **VII**

Borane-dimethyl sulfide complex (7.23 mL, 76.2 mmol) was slowly added to a solution of methyl 5-*tert*-butylhydrogenisophthalate **VI** (12.0 g, 50.8 mmol) in THF (100 ml) over the period of 20 minutes at room temperature. The mixture was stirred for 1.5 hours at room temperature and then refluxed for 1 additional hour. The reaction mixture was then cooled and the unreacted borane was decomposed with methanol (10 mL). The solvents were removed under reduced pressure and the residue was dissolved in ethyl acetate. The organic solution was washed with a saturated $NaHCO_3$ solution, dried over $MgSO_4$, and purified by a silica gel column chromatography (hexane/ethyl acetate) to afford methyl 3-*tert*-butyl-5-hydroxymethylbenzoate **VII** (10.0 g, 45.0 mmol, 90%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.9 (s, 3H), 4.7 (s, 2H), 7.6 (s, 1H), 7.8 (s, 1H), 8.0 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 31.4 (3C), 35.0, 52.3, 65.3, 125.5, 126.1, 128.8, 130.3, 141.0, 152.1, 167.5.

Methyl 3-bromomethyl-5-*tert*-butylbenzoate **VIII**

To a solution of methyl 3-*tert*-butyl-5-hydroxymethylbenzoate **VII** (9.50 g, 42.7 mmol) and carbon tetrabromide (17.25 g, 52.0 mmol) in dichloromethane (50

mL) was slowly added triphenylphosphine (13.6 g, 52.0 mmol) over the period of 20 minutes, and the mixture was stirred at room temperature for 25 minutes. The reaction mixture was concentrated under reduced pressure and the residue was suspended in ethyl acetate. The precipitate was removed by filtration and the filtrate was concentrated. The crude material was purified by a silica gel chromatography (hexanes/ethyl acetate, 4:1), and the product was re-crystallized form ethyl acetate/hexanes to afford methyl 3-bromomethyl-5-*tert*-butylbenzoate **VIII** (12.0 g, 42.1 mmol, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) δ1.45 (s, 9H), 3.7 (s, 3H), 4.4 (s, 2H), 7.6 (s, 1H), 7.8 (s, 1H), 8.0 (s, 1H); ^{13}C NMR ($CDCl_3$) δ31.3 (3C), 33.2, 36.0, 52.3, 126.9, 127.5, 130.6, 130.7, 137.9, 152.4, 167.0.

5-(3-*Tert*-butyl-5-methoxycarbonyl-benzyl)-2,2-dimethyl-5-[3-[(triphenylmethyl)thio]propyl]-[1,3]dioxane-4,6-dione

A solution of methyl 3-bromomethyl-5-*tert*-butylbenzoate (10.3 g, 36.1 mmol), 2,2-dimethyl-5-[3-[(triphenylmethyl)-thio]propyl]-[1,3]dioxane-4,6-dione **IX** (13.8 g, 30.0 mmol), and benzyltriethylammonium chloride (6.38 g, 30 mmol) in acetonitrile (90 mL) was added potassium carbonate (4.35 g, 30 mmol) at 25 °C, and the reaction mixture was stirred at 60 °C overnight (the synthesis of compound **IX** was previously described in International Publication No. WO 00/01668). The solvent was removed under reduced pressure and the residue was

partitioned between ethyl acetate and a 10% aqueous $KHSO_4$ solution. The organic layer was dried over $MgSO_4$, concentrated. The crude material was recrystallized from ethyl acetate/hexane mixture to afford 5-(3-*tert*-butyl-5-methoxycarbonyl-benzyl)-2,2-dimethyl-5-[3-[(triphenyl-methyl)thio]propyl]-[1,3]dioxane-4,6-dione **X** (14.0 g, 79%) as a white solid: ^1H NMR ($CDCl_3$) δ 0.7 (s, 3H), 1.3 (s, 9H), 1.2-1.3 (m, 2H), 1.5 (s, 3H), 2.0 (m, 2H), 2.2 (m, 2H), 3.3 (s, 2H), 3.8 (s, 3H), 7.2-7.4 (m, 16H), 7.6 (s, 1H), 7.8 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 29.1, 29.4, 31.2, 31.4, 34.9, 40.3, 43.7, 52.3, 57.3, 66.8, 105.8, 126.0, 126.8, 128.0, 128.5, 129.6, 130.5, 132.3, 135.3, 144.8, 152.4, 167.1, 168.5.

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2-(3-*tert*-Butyl-5-methoxycarbonyl-benzyl)-2-[3-[(triphenylmethyl)thio]propyl]-malonic acid **XI**

To a solution of 5-(3-*tert*-butyl-5-methoxycarbonylbenzyl)-2,2-dimethyl-5-[3-[(triphenylmethyl)thio]propyl]-[1,3]dioxane-4,6-dione **X** (11 g, 16.5 mmol) in 1,4-dioxane (15 ml) was added a solution of sodium hydroxide (4.63 g, 115.5 mmol) in H_2O (15 mL) at 25 °C, and the mixture was stirred at 100 °C for 1 hour. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and a 10% aqueous $KHSO_4$ solution. The organic layer was dried over $MgSO_4$, concentrated. The crude material was recrystallized from ethyl acetate/hexane mixture to afford 2-(3-*tert*-butyl-5-methoxycarbonyl-benzyl)-2-[3-

[(triphenyl-methyl)thio]-propyl]malonic acid **XI** (9.0 g, 90%) as a white solid: ^{1}H NMR (CD_3OD) δ 1.4 (s, 9H), 1.4 (m, 2H), 1.6 (m, 2H), 2.1 (t, J = 8.0 Hz, 2H), 3.2 (s, 2H), 7.1-7.4 (m, 16H), 7.7 (s, 1H), 7.9 (s, 1H); ^{13}C NMR (CD_3OD) δ 24.8, 31.8 (3C), 32.4, 33.3, 35.6, 39.0, 59.5, 67.7, 126.2, 127.7, 128.9, 129.6, 130.7, 131.5, 132.9, 137.8, 146.2, 152.6, 170.1, 174.5.

2-(3-*Tert*-Butyl-5-methoxycarbonyl-benzyl)-5-[(triphenylmethyl)thio]pentanoic acid **XII**

A solution of 2-(3-*tert*-butyl-5-methoxycarbonyl-benzyl)-2-[3-[(triphenylmethyl)thio]propyl]-malonic acid **XI** (6.71 g, 11 mmol) in DMSO (10 ml) was stirred at 130 °C for 1.5 hours. The solvent was removed under reduced pressure and water was added to the residual oil. The precipitate was filtered off, washed with water, and dried under vacuum to afford 2-(3-*tert*-butyl-5-methoxycarbonyl-benzyl)-5-[(triphenylmethyl)thio]-pentanoic acid **XII** (5.86 g, 10.3 mmol, 94%) as a white solid: ^{1}H NMR (CD_3OD) δ 1.3 (s, 9H), 1.3-1.5 (m, 4H), 2.1 (m, 2H), 2.4 (m, 1H), 2.7 (m, 1H), 2.8 (m, 1H), 7.1-7.4 (m, 16H), 7.7 (s, 1H), 7.9 (s, 1H); ^{13}C NMR (CD_3OD) δ 27.4, 31.7 (3C), 32.3, 32.7, 35.6, 39.2, 48.4, 67.7, 125.7, 127.7, 128.6, 128.9, 130.8, 131.6, 132.0, 140.8, 146.3, 152.7, 170.3, 178.8.

3-Carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid

To a solution of 2-(3-*tert*-butyl-5-methoxycarbonyl-

benzyl)-5-[(triphenylmethyl)thio]pentanoic acid **XII** (5.5 g, 9.7 mmol) in dichloromethane (30 mL) were added triisopropylsilane (2.4 mL, 11.6 mmol) and trifluoroacetic acid (10 mL), and the mixture was stirred at room temperature for 10 minutes. The solvent was removed under reduced pressure and the crude material was purified by silica gel chromatography (1% AcOH in Hexanes/EtOAc, 4:1) to afford 3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid (1.7 g, 5.3 mmol, 55%) as a white solid: ^{1}H NMR (CD_3OD) δ 1.3 (s, 9H), 1.5-1.8 (m, 4H), 2.4 (m, 2H), 2.6-2.7 (m, 1H), 2.8-2.9 (m, 1H), 2.9-3.0 (m, 1H), 7.5 (s, 1H), 7.7 (s, 1H), 7.8 (s, 1H); ^{13}C NMR (CD_3OD) δ 24.8, 31.7 (3C), 31.9, 32.9, 35.6, 39.5, 48.6, 125.7, 128.5, 131.6, 132.0, 140.9, 152.8, 170.3, 179.0. Elemental analysis calculated for $C_{17}H_{24}O_4S$: C, 62.93; H, 7.46; S, 9.88. Found: C, 63.02; H, 7.36; S, 9.82.

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EXAMPLE 6

Preparation of 3-(1-Carboxy-4-mercaptobutoxy)-benzoic Acid

(Scheme VII)

Methyl 3-(4-acetylthio-1-methoxycarbonyl-butoxy)-benzoate **XV**

To a solution of methyl 2,5-dibromopentanoate **XIII** (22.00 g, 80.3 mmol) and methyl 3-hydroxybenzoate **XIV** (10.18 g, 66.9 mmol) in DMF (80 mL) was added K_2CO_3 (12.94 g, 93.7 mmol) at room temperature. The mixture was stirred

at room temperature under N_2 for 12 hours, then heated at 70 °C for 1 hour. Potassium thioacetate (22.93 g, 200.8 mmol) was added to the mixture, which was heated at 70 °C for 2 hours. The mixture was allowed to cool to room temperature and was diluted with EtOAc (1000 mL). The mixture was washed with H_2O (3 X 300 mL) and brine (2 X 300 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (gradient elution: 10% to 20% EtOAc/hexanes) to afford methyl 3-(4-acetylthio-1-methoxycarbonyl-butoxy)-benzoate **XV** (8.40 g, 24.7 mmol, 37%) as a yellow oil: R_f 0.26 (hexanes/EtOAc, 4:1): ^{1}H NMR ($CDCl_3$) δ 1.73-1.88 (m, 2H), 2.01-2.08 (m, 2H), 2.32 (s, 3H), 2.93 (t, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.89 (s, 3H), 4.69 (t, J = 6.2 Hz, 1H), 7.07 (dm, J = 8.0 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.50 (m, 1H), 7.65 (dm, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 25.3, 28.3, 30.5, 31.4, 52.1, 52.2, 75.8, 115.4, 120.0, 122.8, 129.5, 131.4, 157.5, 166.4, 171.3, 195.4.

3-(1-Carboxy-4-mercaptobutoxy)-benzoic acid

A solution of methyl 3-(4-acetylthio-1-methoxycarbonylbutoxy)benzoate **XV** (8.00 g, 23.5 mmol) in THF (60 mL) was deoxygenated by bubbling N_2 through the solution for 1 hour. To the solution was added deoxygenated 3 N NaOH (47 mL, 141 mmol) and the mixture was stirred for 24 hours at room temperature under N_2. The mixture was acidified with 1 N HCl and extracted with

EtOAc (3 X 300 mL). The organic extracts were washed with water (300 mL) and brine (300 mL), dried over $MgSO_4$, filtered, and concentrated. The residual oil was dissolved in ether and the solution was concentrated to afford 3-(1-carboxy-4-mercaptobutoxy)-benzoic acid (4.40 g, 16.3 mmol, 69%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.40 (t, J = 7.9 Hz, 1H), 1.83-1.99 (m, 2H), 2.11-2.22 (m, 2H), 2.63 (m, 2H), 4.76 (dd, J = 7.3, 5.0 Hz, 1H), 7.23 (m, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.49 (m, 1H), 7.72 (d, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 24.1, 29.6, 31.2, 75.5, 114.8, 122.1, 123.9, 129.9, 130.5, 157.6, 171.8, 177.1. Elemental analysis calculated for $C_{12}H_{14}O_5S$: C, 53.32; H, 5.22; S, 11.86. Found: C, 53.04; H, 5.38; S, 11.58.

EXAMPLE 7

Preparation of (+)-3-carboxy-α-(3-mercaptopropyl)-benzenepropanoic acid 53 and (-)-3-carboxy-α-(3-mercaptopropyl)-benzenepropanoic acid

A solution of racemic 3-carboxy-α-(3-mercaptopropyl)benzenepropanoic acid (2.70 g, 10.1 mmol) was divided into multiple samples of equal volume, and each of them was passed through a CHIRAPAK AD column (250 mm x 21 mm id) using carbon dioxide/methanol (77/23, v/v) as eluent at a flow rate of 25 mL/min, at 25 °C. The first eluting peak (detected by UV at 290 nm) from each run was combined and concentrated to afford (+)-3-carboxy-

α-(3-mercaptopropyl)-benzenepropanoic acid (1.02 g, 38%) as a colorless oil: $[\alpha]^{25}D$ = +15.5 (*c* = 1.1, CH3CN). (-)-3-carboxy-α-(3-mercaptopropyl)-benzenepropanoic acid (1.08 g. 40%) was obtained likewise from the second peaks as a colorless oil: $[\alpha]^{25}D$ = -13.4 (*c* = 1.1, CH_3CN).

EXAMPLE 8

Preparation 3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic Acid (Scheme VIII)

3-(2,2-Dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester

To a solution of 2,2-dimethyl-5-trifluoromethanesulfonyloxy-4*H*-1,3-benzodioxin-4-one (2.0 g, 5.8 mmol), 3-ethoxycarbonylphenylboronic acid (1.34 g, 6.9 mmol) and anhydrous K_2CO_3 powder (2.61 g, 18.9 mmol) in DMF (30 mL) was added tetrakis(triphenylphosphine) palladium(0.202 g, 0.175 mmol). The mixture was heated at reflux for 2 hours. The reaction mixture was allowed to cool to room temperature and 1 N HCl (25 mL) was added. The mixture was extracted with EtOAc (3 X 25 mL). The combined extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography (1:15 EtOAc/hexanes) to afford 3-(2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester (1.2 g, 63%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.39 (t, *J* = 7.1 Hz,

3H), 1.80 (s, 6H), 4.39 (q, J = 7.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 7.47-7.57 (m, 3H), 8.00 (t, J = 1.5 Hz, 1H), 8.07 (dt, J = 7.5, 1.5 Hz, 1H).

3-Hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-(2,2-dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)benzoic acid, ethyl ester (1.4 g, 4.3 mmol) in methanol (10 mL) was added sodium methoxide (0.5 M in methanol, 25 mL) at 0 °C. The solution was stirred at room temperature for 15 minutes. The reaction was quenched by addition of 1 N HCl (30 mL) and extracted with EtOAc (3 X 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to afford 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.2 g, 95%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H), 3.93 (s, 3H), 6.79 (dd, J = 7.5, 0.9 Hz, 1H), 7.04 (dd, J = 7.5, 0.9 Hz, 1H), 7.43 (m, 3H), 7.93 (m, 1H), 8.02 (dm, J = 7.0 Hz, 1H), 10.8 (s, 1H).

3-Trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.1 g, 3.8 mmol) in dichloromethane (15 mL) were added pyridine (1.00 mL, 12.3 mmol) and trifluromethanesulfonic anhydride (0.90 mL, 5.4 mmol) at 0 °C. The solution was stirred at 0 °C for 2 hours. Aqueous 1 N HCl (20 mL) was added, and the mixture

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was extracted with dichloromethane (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.4 g, 87%) as a yellow solid: ^1H NMR ($CDCl_3$) δ 3.72 (s, 3H), 3.94 (s, 3H), 7.38-7.62 (m, 5H), 8.08 (m, 2H).

3-Ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

A mixture of 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.3 g, 3.1 mmol), tetrakis(triphenylphosphine)palladium (0.36 g, 0.31 mmol), LiCl (0.94 g, 22.2 mmol), triethylamine (0.6 mL, 4.3 mmol) and tri-n-butyl(vinyl)tin (1.0 mL, 3.4 mmol) in 1,4-dioxane (30 mL) was heated at reflux under N_2 for 4 hours. After cooling to room temperature, the mixture was filtered through a plug of silica gel and the filtrate was concentrated. Purification by flash chromatography (1:10 EtOAc/hexanes) provided 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.91 g, 99%) as a white solid: ^1H NMR ($CDCl_3$) δ 3.61 (s, 3H), 3.92 (s, 3H), 5.40 (d, J = 11.1 Hz, 1H), 5.79 (d, J = 17.5 Hz, 1H), 6.87 (dd, J = 17.4, 11.0 Hz, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.44-7.49 (m, 2H), 7.56 (dm, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.03 (dm, J = 7.5 Hz, 1H), 8.08 (t, 1, J = 1.5 Hz, 1H).

3-[2-(Acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.85 g, 2.9 mmol) in benzene (10 mL) was added thioacetic acid (2.1 mL, 29.4 mmol) followed by AIBN (0.053 g, 0.32 mmol). The solution was deoxygenated for 30 minutes by bubbling nitrogen through the solution and then heated at reflux for 4 hours. Saturated aqueous $NaHCO_3$ (20 mL) was added to the solution and the mixture was extracted with EtOAc (2 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:12 EtOAc/hexanes) to give 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.51 g, 48%) as an off white solid: ^1H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.93 (m, 2H), 3.14 (m, 2H), 3.62 (s, 3H), 3.93 (s, 3H), 7.29 (dd, J = 7.6, 0.9 Hz, 1H), 7.35 (dd, J = 7.5, 0.8 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.55 (dt, J = 8.0, 1.5 Hz, 1H), 8.03 (dt, J = 7.9, 1.5 Hz, 1H), 8.07 (t, J = 1.5 Hz, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester

To a deoxygenated solution of 3-[2-(acetylthio) ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.50 g, 1.34 mmol) in THF (3.5 mL) was added a

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deoxygenated solution of NaOH (0.38 g, 9.4 mmol) in water (3.5 mL). The mixture was stirred overnight, and 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.35 g, 83%) as an off white solid: ^1H NMR ($CDCl_3$) δ 1.46 (t, J = 8.0 Hz, 1H), 2.83 (m, 2H), 3.00 (m, 2H), 3.60 (s, 3H), 7.33-7.31 (m, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.61 (dm, J = 7.9 Hz, 1H), 8.10 (dm, J = 7.9 Hz, 1H), 8.14 (m, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid

To a deoxygenated suspension of sodium ethanethiolate (0.135 g, 1.60 mmol) in DMF (0.5 mL) was added a solution of 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.10 g, 0.32 mmol) in DMF (0.5 mL). Argon was bubbled through the mixture for 10 minutes. The reaction was heated at 100 °C for 1 hour and 200 °C for another hour. After the mixture cooled to room temperature, the reaction was quenched with 1 N HCl (20 mL) and was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.055 g, 57%) as a white solid: ^1H NMR

($CDCl_3$) δ 1.51 (t, *J* = 8.0 Hz, 1H), 2.87-2.93 (m, 2H), 3.12-3.08 (m, 2H), 7.37 (m, 2H), 7.57-7.47 (m, 2H), 7.70 (dm, *J* = 7.9 Hz, 1H), 7.98 (dm, *J* = 7.8 Hz, 1H), 8.30 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 26.2, 38.8, 128.1, 129.3, 129.7, 129.8, 129.9 (2C), 130.5, 133.3, 134.4, 137.7, 139.1, 141.2, 172.3, 176.9. Elemental analysis calculated for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67; S, 10.61. Found: C, 63.65; H, 4.88; S, 10.33.

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EXAMPLE 9

Preparation of 2-[(4-carboxyphenyl)methoxy]-6-(2-`mercaptoethyl)- benzoic Acid

(Scheme IX)

5-Ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one

A mixture of 2,2-dimethyl-5-trifluoromethanesulfonyloxy-4*H*-1,3-benzodioxin-4-one (9.90 g, 30.3 mmol), tributyl(vinyl)tin (10.10 g, 31.9 mmol), lithium chloride (8.70 g, 205 mmol), and triethylamine (5.0 mL, 36.0 mmol) in 1,4-dioxane (300 mL) was deoxygenated by bubbling nitrogen through the mixture for 1 hour. To the mixture was added tetrakis(triphenylphosphine)palladium (3.40 g, 2.90 mmol) and the mixture was heated at 100 °C for 3 hours. The mixture was allowed to cool to room temperature and was filtered. The filtrate was concentrated and purified by flash chromatography (1:12, EtOAc/hexanes) to provide 5-ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (5.00 g,

81%) as a yellow oil: ^{1}H NMR: ($CDCl_3$) δ 1.72 (s, 6H), 5.43 (dd, *J* = 11.0, 1.3 Hz, 1H), 5.72 (dd, *J* = 17.5, 1.3 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.73 (dd, *J* = 17.5, 11.0 Hz, 1H).

2-Ethenyl-6-hydroxybenzoic acid, methyl ester

To 5-ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (4.01 g, 19.6 mmol) was added 0.5 M sodium methoxide in methanol (85 mL, 42.5 mmol) at room temperature. Aqueous 1 N HCl (100 mL) was added to the solution after 15 minutes. The cloudy solution was extracted with ether (2 X 100 mL). The combined organic extracts were washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated to afford 2-ethenyl-6-hydroxybenzoic acid, methyl ester (2.0 g, 57%) as a yellow oil. This material was used without further purification in the next step: ^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H), 5.26 (dd, *J* = 10.8, 1.5 Hz, 1H), 5.49 (dd, *J* = 17.3, 1.5 Hz, 1H), 6.95 (m, 2H), 7.23-7.39 (m, 2H), 11.12 (s, 1H).

2-Ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester

To a stirred solution of the above material (0.500 g, 2.8 mmol) in acetone (10 mL) were added K_2CO_3 (1.50 g, 10.9 mmol) and methyl 4-(bromomethyl)benzoate (0.71 g, 3.10 mmol) at room temperature. The mixture was stirred under nitrogen for 3 hours and filtered. The filtrate was concentrated and residue was purified by flash

chromatography (1:10 EtOAc/hexanes) to provide 2-ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.73 g, 80%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 6H), 5.17 (s, 2H), 5.37 (dd, J = 11.1, 1.0 Hz, 1H), 5.78 (dd, J = 17.6, 0.9 Hz, 1H), 6.70 (dd, 1, J = 17.4, 11.1 Hz, 1H), 6.83 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H) 7.29 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H).

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2-[2-(Acetylthio)ethyl]-6-[4-(methoxycarbonyl)phenyl]-methoxy-benzoic acid, methyl ester

To a solution of 2-ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.71 g, 2.18 mmol) in benzene (10 mL) was added thioacetic acid (1.80 mL, 25.2 mmol) followed by AIBN (37 mg, 0.23 mmol). After nitrogen was bubbled through the solution for 30 minutes, the solution was heated at reflux for 4 hours. The reaction was allowed to cool to room temperature and saturated $NaHCO_3$ (20 mL) was added. The mixture was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered and concentrated in *vacuo*. The residue was purified by flash chromatography (1:10 EtOAc/hexanes) to give 2-[2-(acetylthio)ethyl]-6-[4-(methoxycarbonyl)-phenyl]methoxy-benzoic acid, methyl ester (0.50 g, 60%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.85-2.82 (m, 2H), 3.07-3.10 (m, 2H), 3.92 (s,

3H), 3.94 (s, 3H), 5.16 (s, 2H), 6.81 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 7.7 Hz, 1H), 7.28 (t, J = 8.2 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H).

2-[(4-Carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid

To a deoxygenated solution of 2-[2-(acetylthio)ethyl]-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.20 g, 0.50 mmol) in 95% EtOH (3 mL) was added a deoxygenated solution of KOH (0.463 g, 8.3 mmol) in 95% EtOH (3 mL) under nitrogen. The solution was heated at reflux overnight and quenched by addition of 1 N HCl (20 mL). The mixture was extracted with EtOAc (3 x 20 mL) and the combined organic extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. Purification by flash chromatography (1:1 dichloromathane/hexanes with 1% acetic acid) provided 2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid (0.077 g, 46%) as a white solid: ^{1}H NMR (CD_3OD) δ 2.75 (m, 2H), 2.92 (m, 2H), 5.22 (s, 2H), 6.93 (d, J = 7.5 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 7.30 (t, J = 8.3 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H); ^{13}C NMR (CD_3OD) δ 26.5, 39.8, 71.1, 112.4, 123.9, 126.9, 128.3, 131.3, 131.7, 131.8, 139.9, 144.2, 156.7, 170.0, 172.3. Elemental analysis calculated for $C_{17}H_{16}O_5S$: C, 61.43; H, 4.85; S, 9.65. Found: C, 61.16; H, 4.95; S, 9.44.

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EXAMPLE 10

Preparation of 4-mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (Scheme X)

4-Methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid

To a solution of 2-bromo-5-methylbenzoic acid (5.00 g, 23.3 mmol) in DME (100 mL) were added 3-carboxyphenylboronic acid (3.86 g; 23.3 mmol), a solution of Na_2CO_3 (9.90 g, 93 mmol) in H_2O and tetrakis(triphenylphosphine)palladium. The mixture was stirred at 90 °C for 4 days. The mixture was allowed to cool to room temperature, diluted with EtOAc (50 mL), and washed with a saturated $NaHCO_3$ solution. The aqueous layer was separated, acidified with 10 % HCl, and extracted with EtOAc (3 X 20 mL). The combined extracts were dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (9:1 hexanes/EtOAc 1% acetic acid) to afford 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (2.20 g, 37 %) as a solid: ^1H NMR (DMSO-d_6) δ 2.40 (s, 3H), 7.30 (m, 1H), 7.42 (m, 1H), 7.52-7.57 (m, 2H), 7.60 (s, 1H), 7.85 (s, 1H), 7.91-7.92 (m, 1H).

4-Methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (2.20 g, 8.6 mmol) in methanol (150 mL) was added conc. H_2SO_4 (1.6 mL) and the mixture was heated at reflux overnight. The solvent was removed under a reduced pressure and the residue was partitioned between

saturated aqueous $NaHCO_3$ solution and EtOAc (20 mL). The organic layer was dried over $MgSO_4$ and concentrated to give 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (2.26 g, 92%) as a crude material. This product was used for the next reaction without further purification: ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H), 3.58 (s, 3H), 3.88 (s, 3H), 7.36-7.38 (m, 1H), 7.47-7.48 (m, 1H), 7.56-7.58 (m, 2H), 7.62 (s, 1H), 7.82 (s, 1H), 7.94-7.96 (m, 1H).

4-Bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (2.26 g, 7.9 mmol) in CCl_4 (50 mL) were added benzoyl peroxide (0.010 g, 0.04 mmol) and NBS (1.42 g, 8.0 mmol), and the mixture was refluxed for 3 days. The reaction mixture was allowed to cool to room temperature, filtered, and concentrated. The residue was purified by column chromatography (95:5 to 90:10 hexanes/EtOAc) to afford 4-bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.71 g, 60 %): ^1H NMR (DMSO-d_6) δ 3.61(s, 3H), 3.88 (s, 3H), 4.84 (s, 2H), 7.48-7.50 (d, J = 8.0 Hz, 1H), 7.59-7.60 (m, 2H), 7.72-7.75 (m, 1H), 7.85 (s, 1H), 7.90 (m, 1H), 7.97-7.99 (m, 1H).

4-Acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 4-bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.59 g, 4.4 mmol) in acetone (75 mL) was added potassium thioacetate (0.60 g,

5.3 mmol), and the mixture was refluxed for 1 hour. The mixture was allowed to cool to room temperature, filtered, and concentrated. The residual product was purified by column chromatography (hexanes/EtOAc, 9/1) to afford 4-acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.21 g, 76%): ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 3.60 (s, 3H), 3.88 (s, 3H), 4.23 (s, 2H), 7.42-7.44 (d, J = 8.0 Hz, 1H), 7.57-7.60 (m, 3H), 7.74 (s 1H), 7.83 (s, 1H), 7.96-7.99 (m, 1H).

4-Mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid

To a solution of 4-acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.27 g, 0.75 mmol) in deoxygenated THF was added a degassed solution of sodium hydroxide (0.12 g, 3.0 mmol) in H_2O (5 mL) at room temperature. After 24 hours, additional solution of sodium hydroxide (0.09 g) in H_2O (2 mL) was added to the reaction mixture and the mixture was stirred for 24 hours. The mixture was acidified with 10 % HCl and extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography with (9:1 dichloromethane/EtOAc with 1% acetic acid) to afford 4-mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.20 g, 92%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.10 (t, J = 8.03 Hz, 1H), 3.89 (d, J = 8.03 Hz, 2H), 7.43 (d, J = 7.53 Hz, 1H), 7.58-7.65 (m, 3H), 7.83 (d, J = 2.01 Hz, 1H), 7.92 (s, 1H), 7.97-8.00 (m, 1H). Elemental analysis calculated for $C_{15}H_{12}O_4S \cdot 0.5$

AcOH: C, 60.37; H, 4.43; O, 25.13; S, 10.07. Found: C, 60.28; H, 4.45; S, 10.15.

EXAMPLE 11

Preparation of Precursor Compound 3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic Acid (Scheme XVIII)

3-(2,2-Dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester

To a solution of 2,2-dimethyl-5-trifluoromethanesulfonyloxy-4*H*-1,3-benzodioxin-4-one (2.0 g, 5.8 mmol), 3-ethoxycarbonylphenylboronic acid (1.34 g, 6.9 mmol) and anhydrous K_2CO_3 powder (2.61 g, 18.9 mmol) in DMF (30 mL) was added tetrakis(triphenylphosphine)palladium (0.202 g, 0.175 mmol). The mixture was heated at reflux for 2 h. The reaction mixture was allowed to cool to room temperature (" rt") and 1 N HCl (25 mL) was added. The mixture was extracted with EtOAc (3 x 25 mL). The combined extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography (1:15 EtOAc/hexanes) to afford 3-(2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester (1.2 g, 63%) as a white solid: ^{1}H NMR ($CDCl_3$) 1.39 (t, J = 7.1 Hz, 3H), 1.80 (s, 6H), 4.39 (q, J = 7.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 7.47-7.57 (m, 3H), 8.00 (t, J = 1.5 Hz, 1H), 8.07 (dt, J = 7.5, 1.5 Hz, 1H).

3-Hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-(2,2-dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)benzoic acid, ethyl ester (1.4 g, 4.3 mmol) in methanol (10 mL) was added sodium methoxide (0.5 M in methanol, 25 mL) at 0 °C. The solution was stirred at rt for 15 min. The reaction was quenched by addition of 1 N HCl (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to afford 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.2 g, 95%) as a yellow solid: ^1H NMR ($CDCl_3$) 3.43 (s, 3H), 3.93 (s, 3H), 6.79 (dd, J = 7.5, 0.9 Hz, 1H), 7.04 (dd, J = 7.5, 0.9 Hz, 1H), 7.43 (m, 3H), 7.93 (m, 1H), 8.02 (dm, J = 7.0 Hz, 1H), 10.8 (s, 1H).

3-Trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.1 g, 3.8 mmol) in dichloromethane (15 mL) were added pyridine (1.00 mL, 12.3 mmol) and trifluromethanesulfonic anhydride (0.90 mL, 5.4 mmol) at 0 °C. The solution was stirred at 0 °C for 2 h. Aqueous 1 N HCl (20 mL) was added, and the mixture was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-

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dicarboxylic acid, dimethyl ester (1.4 g, 87%) as a yellow solid: ^{1}H NMR ($CDCl_3$) 3.72 (s, 3H), 3.94 (s, 3H), 7.38-7.62 (m, 5H), 8.08 (m, 2H).

3-Ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

A mixture of 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.3 g, 3.1 mmol), tetrakis(triphenylphosphine)palladium (0.36 g, 0.31 mmol), LiCl (0.94 g, 22.2 mmol), triethylamine (0.6 mL, 4.3 mmol) and tri-n-butyl(vinyl)tin (1.0 mL, 3.4 mmol) in 1,4-dioxane (30 mL) was heated at reflux under N_2 for 4 h. After cooling to rt, the mixture was filtered through a plug of silica gel and the filtrate was concentrated. Purification by flash chromatography (1:10 EtOAc/hexanes) provided 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.91 g, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) 3.61 (s, 3H), 3.92 (s, 3H), 5.40 (d, J = 11.1 Hz, 1H), 5.79 (d, J = 17.5 Hz, 1H), 6.87 (dd, J = 17.4, 11.0 Hz, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.44-7.49 (m, 2H), 7.56 (dm, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.03 (dm, J = 7.5 Hz, 1H), 8.08 (t, 1, J = 1.5 Hz, 1H).

3-[2-(Acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

To a solution of 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.85 g, 2.9 mmol) in benzene (10 mL) was added thioacetic acid (2.1 mL, 29.4 mmol) followed by AIBN (0.053 g, 0.32 mmol). The solution was deoxygenated for 30 min by bubbling nitrogen through

the solution and then heated at reflux for 4 h. Saturated aqueous $NaHCO_3$ (20 mL) was added to the solution and the mixture was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:12 EtOAc/hexanes) to give 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.51 g, 48%) as an off white solid: ^{1}H NMR ($CDCl_3$) 2.35 (s, 3H), 2.93 (m, 2H), 3.14 (m, 2H), 3.62 (s, 3H), 3.93 (s, 3H), 7.29 (dd, J = 7.6, 0.9 Hz, 1H), 7.35 (dd, J = 7.5, 0.8 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.55 (dt, J = 8.0, 1.5 Hz, 1H), 8.03 (dt, J = 7.9, 1.5 Hz, 1H), 8.07 (t, J = 1.5 Hz, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester

To a deoxygenated solution of 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.50 g, 1.34 mmol) in THF (3.5 mL) was added a deoxygenated solution of NaOH (0.38 g, 9.4 mmol) in water (3.5 mL). The mixture was stirred overnight, and 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.35 g, 83%) as an off white solid: ^{1}H NMR ($CDCl_3$) 1.46 (t, J =

8.0 Hz, 1H), 2.83 (m, 2H), 3.00 (m, 2H), 3.60 (s, 3H), 7.33-7.31 (m, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.61 (dm, J = 7.9 Hz, 1H), 8.10 (dm, J = 7.9 Hz, 1H), 8.14 (m, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid

To a deoxygenated suspension of sodium ethanethiolate (0.135 g, 1.60 mmol) in DMF (0.5 mL) was added a solution of 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.10 g, 0.32 mmol) in DMF (0.5 mL). Argon was bubbled through the mixture for 10 min. The reaction was heated at 100 °C for 1 h and 200 °C for another hour. After the mixture cooled to rt, the reaction was quenched with 1 N HCl (20 mL) and was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.055 g, 57%) as a white solid: ^{1}H NMR ($CDCl_3$) 1.51 (t, J = 8.0 Hz, 1H), 2.87-2.93 (m, 2H), 3.12-3.08 (m, 2H), 7.37 (m, 2H), 7.57-7.47 (m, 2H), 7.70 (dm, J = 7.9 Hz, 1H), 7.98 (dm, J = 7.8 Hz, 1H), 8.30 (m, 1H); ^{13}C NMR ($CDCl_3$) 26.2, 38.8, 128.1, 129.3, 129.7, 129.8, 129.9 (2C), 130.5, 133.3, 134.4, 137.7, 139.1, 141.2, 172.3, 176.9. Anal. Calcd for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67; S, 10.61. Found: C, 63.65; H, 4.88; S, 10.33.

EXAMPLE 12

***In Vitro* Inhibition of NAALADase Activity**

Various compounds used in the inventive methods and pharmaceutical compositions have been tested for *in vitro* inhibition of NAALADase activity. The experimental protocol and some of the results are set forth in U.S. Patents Nos. 5,672,592, 5,795,877, 5,863,536, 5,880,112, 5,902,817, 5,962,521, 5,968,915, 6,025,344, 6,025,345, 6,028,216, 6,046,180, 6,054,444, 6,071,965 and 6,121,252, allowed U.S. Patent Application No. 09/228,391 for which the issue fee has been paid, copending U.S. Patent Application No. 09/438,970 filed November 12, 1999 (corresponding to International Patent Application No. PCT/US00/30977 filed November 13, 2000), and International Publications Nos. WO 99/33849, WO 00/01668 and WO 01/14390, the entire contents of which patents, patent application and publications are herein incorporated by reference, as though set forth herein in full.

Other exemplary results are provided below in TABLES II and III).

TABLE II

***IN VITRO* INHIBITION OF NAALADASE ACTIVITY**

Compound	K_1 (nM)
4-[4-(2,4-dicarboxybenzoyl)phenoxy]-1,2-benzenedicarboxylic acid	1170
2-[(4-carboxyphenyl)sulfonyl]-1,4-benzenedicarboxylic acid	2370
2-[(2,5-dicarboxyphenyl)sulfonyl]-1,4-benzenedicarboxylic acid	1870
4-[(2-carboxyphenyl)thio]-1,3-benzenedicarboxylic acid	3980
2-[(2-carboxyphenyl)thio]-1,4-benzenedicarboxylic acid	572
4-[3-[[3-(2,4-dicarboxyphenoxy)-propyl]-dithio]propoxy]-1,3-benzenedicarboxylic acid	3750
5-(3-mercaptopropoxy)-1,3-benzenedicarboxylic acid	3300
5-(2-mercaptoethoxy)-1,3-benzenedicarboxylic acid	14500
5-[(hydroxyamino)-carbonyl]-1,3-benzenedicarboxylic acid	1000
5-phosphono-1,3-benzenedicarboxylic	14000

Compound	K_1 (nM)
acid	
5-mercaptomethyl-1,3-benzenedicarboxylic acid	6500
5-phosphonomethyl-1,3-benzenedicarboxylic acid	3100
5-[(carboxymethyl)amino]-1,3-benzenedicarboxylic acid	100000
5-[[(2-furanylmethyl)amino]methyl]-1,3-benzenedicarboxylic acid	50000
2-carboxymethyl-1,4-benzenedicarboxylic acid	9000
5-[2-(hydroxyamino)-2-oxoethyl]-1,3-benzenedicarboxylic acid	12000
4-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid	116
5-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid	5100

EDOCID: WO 2004026230A1

TABLE III

***IN VITRO* INHIBITION OF NAALADASE ACTIVITY**

Compound	IC_{50}
alpha-(3-mercaptopropyl)-3-(trifluoromethyl)-benzenepropanoic acid	4000
alpha-(3-mercaptopropyl)-benzenepropanoic acid	2270
4-hydroxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2140
2,3,4,5,6-pentafluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1000
3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	17
4-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	59
alpha-(3-mercaptopropyl)-4-(methylsulfonyl)-benzenepropanoic acid	211
2-cyano-alpha-(3-mercaptopropyl)-benzenepropanoic acid	450
5-(2-carboxy-5-mercaptopentyl)-1,3-benzenedicarboxylic acid	2.55

Compound	IC_{50}
5-carboxy-2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2.09
3-carboxy-4-fluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	12
4-(2-cyanophenyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	316
2-(aminocarbonyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3950
3-(1-carboxy-4-mercaptobutoxy)-benzoic acid	16.3
5-mercapto-2-phenoxy-pentanoic acid	555
2-(3,5-dimethoxyphenoxy)-5-mercapto-pentanoic acid	16,100
alpha-(3-mercaptopropyl)-2,5-dimethoxy-benzenepropanoic acid	566
alpha-(3-mercaptopropyl)-3-phenoxy-benzenepropanoic acid	308
2-(3-hydroxyphenoxy)-5-mercapto-pentanoic acid	846

Compound	IC_{50}
3-(1-carboxy-4-mercaptobutoxy)-benzeneacetic acid	64
4-(1-carboxy-4-mercaptobutoxy)-benzeneacetic acid	82
alpha-(3-mercaptopropyl)-4-phenyl-benzenepropanoic acid	229
2-(3-acetylphenoxy)-5-mercapto-pentanoic acid	2900
2-[3-(acetylamino)phenoxy]-5-mercapto-pentanoic acid	27700
2-(4-acetylphenoxy)-5-mercaptopentanoic acid	29.8
4-(acetylamino)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2200
3-(1-carboxy-4-mercaptobutoxy)-4-methoxy-benzoic acid	287
4-(carboxymethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	809
2-(1-carboxy-4-mercaptobutoxy)-benzoic acid	4600

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Compound	IC_{50}
4-(1-carboxy-4-mercaptobutoxy)-benzoic acid	192
3-carboxy-2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1200
3-carboxy-4-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	175
3-(1-carboxy-4-mercaptobutoxy)-4-chloro-benzoic acid	118
3-(1-carboxy-4-mercaptobutoxy)-4-fluoro-benzoic acid	400
5-carboxy-2-fluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	6.33
5-carboxy-alpha-(3-mercaptopropyl)-2-methoxy-benzenepropanoic acid	26
4-carboxy-alpha-(3-mercaptopropyl)-1-naphthalenepropanoic acid	316
2-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1700
4-carboxy-2,3,5,6-tetrafluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	660

Compound	IC_{50}
5-mercapto-2-(phenylthio)-pentanoic acid	297
3-[1-carboxy-4-mercaptobutyl)thio]-benzoic acid	24
alpha-(3-mercaptopropyl)-2-naphthalenepropanoic acid	1130
2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1350
alpha-(3-mercaptopropyl)-3-[[(phenylmethyl)amino]carbonyl]-benzenepropanoic acid	1130
3-bromo-5-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2.5
3-[[(carboxymethyl)amino]carbonyl]-alpha-(3-mercaptopropyl)-benzenepropanoic acid	286
3-bromo-4-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	146
3-carboxy-alpha-(3-mercaptopropyl)-5-nitro-benzenepropanoic acid	3

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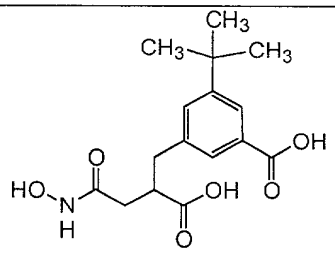
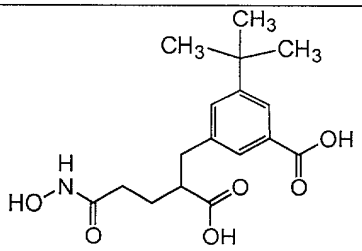
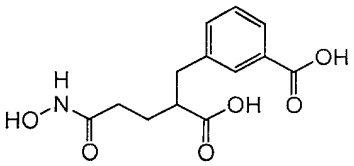
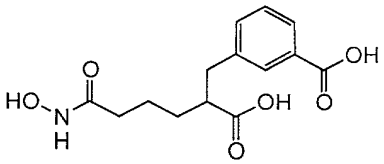
Compound	IC_{50}
3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	0.05
5-carboxy-alpha-(3-mercaptopropyl)-2-nitro-benzenepropanoic acid	8.25
3'-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-3-carboxylic acid	1.83
2-bromo-5-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3.33
(+)-3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	7
(-)-3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	33.3
2-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-4-carboxylic acid	19
6-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	70
4-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	18
3-carboxy-alpha-(3-mercaptopropyl)-5-methoxy-benzenepropanoic acid	13

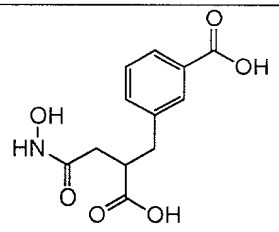
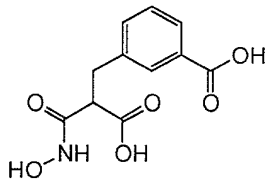
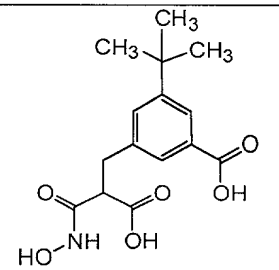
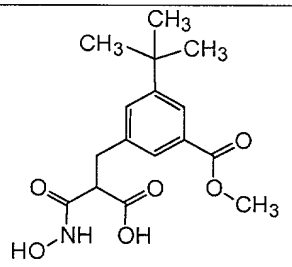
Compound	IC_{50}
3'-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	184
3-(2-carboxyphenoxy)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	49
4-(2-carboxyphenoxy)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3590
3-carboxy-alpha-(3-mercaptobutyl)-benzenepropanoic acid	30
4'-(2-carboxy-5-mercaptopropyl)-[1,1'-biphenyl]-2-carboxylic acid	87
alpha-(3-mercaptopropyl)-3-phenyl-benzenepropanoic acid	181
3-carboxy-alpha-(3-mercaptopropyl)-5-phenoxy-benzenepropanoic acid	5
3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptobutyl)-benzenepropanoic acid	1
2-(2-mercaptoethyl)-benzoic acid	613
5-hydroxy-2-(2-mercaptoethyl)-benzoic acid	9170
5-[(4-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid	71.5

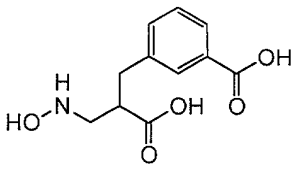
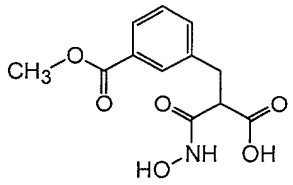
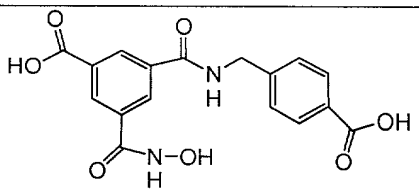
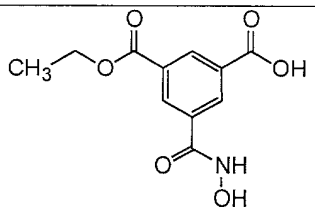
Compound	IC_{50}
2-(2-mercaptoethyl)-5-(phenylmethoxy)-benzoic acid,	380
2-(carboxymethoxy)-6-(2-mercaptoethyl)-benzoic acid	215
5-[(3-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid	84.5
2-(2-mercaptoethyl)-6-(phenylmethoxy)-benzoic acid	11.5
2-[(2-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	11.5
2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	4
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid	3.46
5-(mercaptomethyl)-2-(2-phenylethoxy)-benzoic acid	188
2-(3,3-dimethylbutoxy)-6-(2-mercaptoethyl)-benzoic acid	2580
2-(2-mercaptoethyl)-6-(2-phenylethoxy)-benzoic acid	266
2-[(2-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	160
2-[[3-carboxy-5-(1,1-dimethylethyl)phenyl]methoxy]-6-(2-mercaptoethyl)-benzoic acid	23.7

Compound	IC_{50}
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,4'-dicarboxylic acid	4.28
2-[(4-carboxy-2-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	7
2-[(4-carboxy-3-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	10.5
2-[(2-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	2.65
2-[(3-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	0.098
2-(2-mercaptoethyl)-6-phenoxy-benzoic acid	1150
4-(mercaptomethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid	76
5-(mercaptomethyl)-2-(phenylmethoxy)-benzoic acid	85
4-bromo-3-(mercaptomethyl)-benzoic acid	3200
3-(2-mercaptoethyl)-benzoic acid	6050
3-(mercaptomethyl)-benzoic acid	3780
2-(mercaptomethyl)-benzoic acid	100000

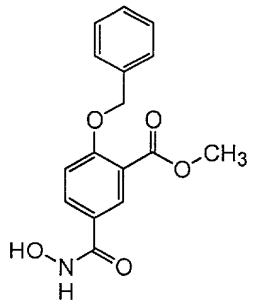
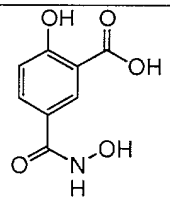
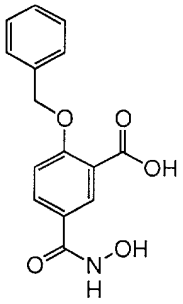
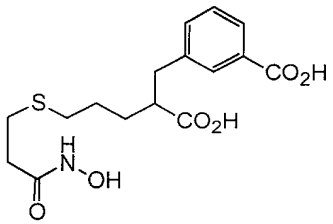
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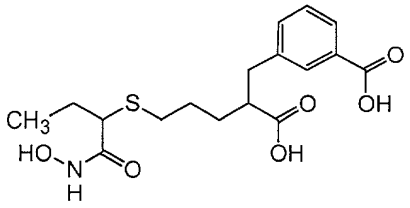
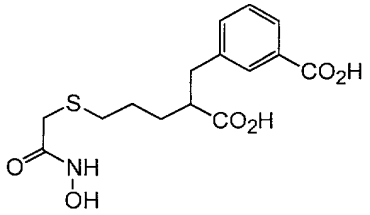
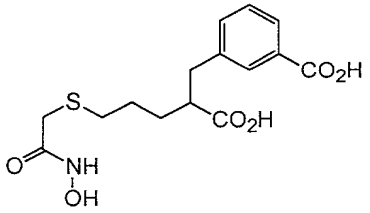
Compound	IC_{50}
 3-*tert*-Butyl-5-(2-carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid	296.3
 3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid	0.2
 3-(2-Carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid	47.8
 3-(2-Carboxy-5-hydroxycarbamoyl-pentyl)-benzoic acid	66.3

Compound	IC_{50}
 3-(2-Carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid	693
 3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid	4530
 3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid	11900
 3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester	38600

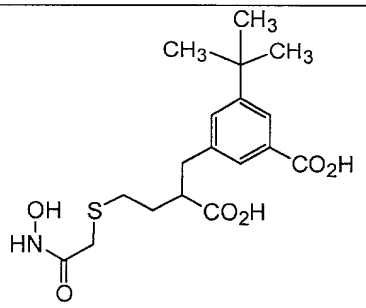
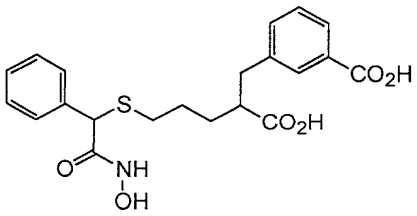
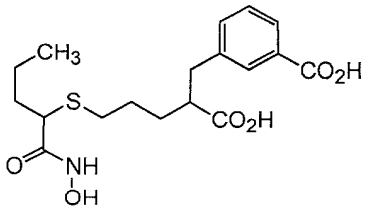
Compound	IC_{50}
 3-(2-Carboxy-3-hydroxyamino-propyl)-benzoic acid	44000
 3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester	100000
	2780
 5-Hydroxycarbamoyl-isophthalic acid monoethyl ester	29000

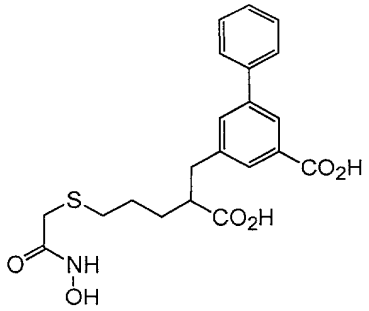
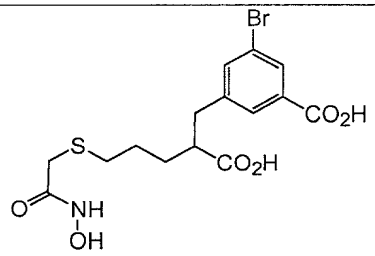
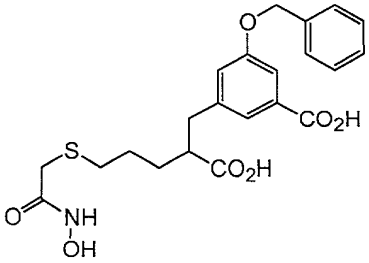
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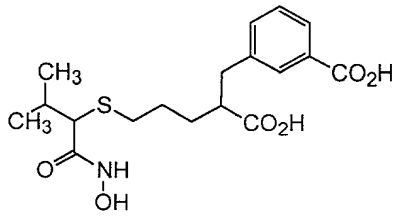
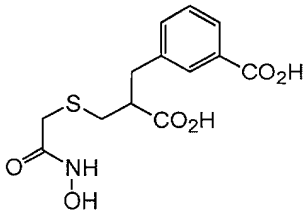
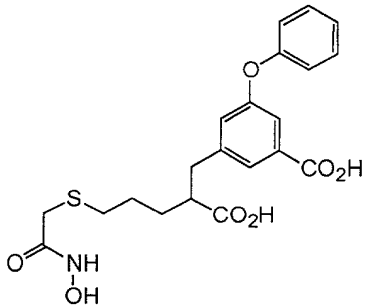
Compound	IC_{50}
 6-Benzyloxy-N-hydroxy-isophthalamic acid methyl ester	100000
 6,N-Dihydroxy-isophthalamic acid	100000
 6-Benzyloxy-N-hydroxy-isophthalamic acid	100000
 3-[2-Carboxy-5-(2-hydroxycarbamoyl-ethylsulfanyl)-pentyl]-benzoic acid	113

Compound	IC_{50}
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-propylsulfanyl)-pentyl]-benzoic acid	3291
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid	384
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanyl-pentyl)-benzoic acid	394

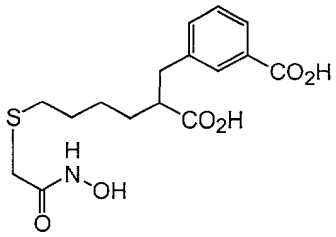
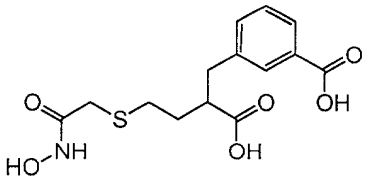
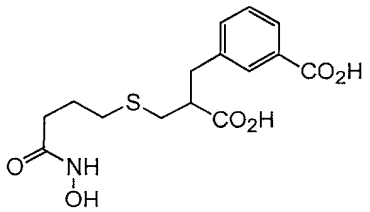
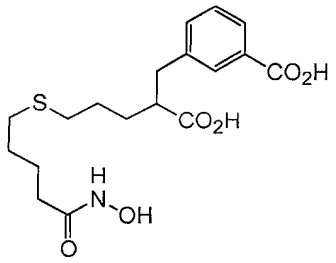
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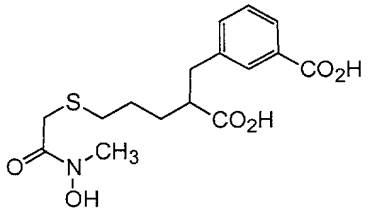
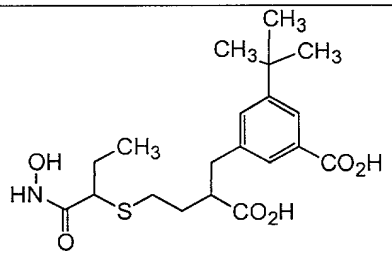
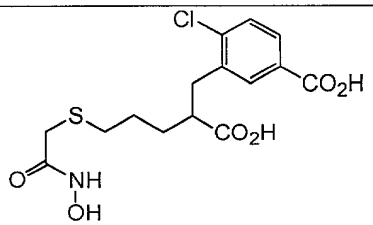
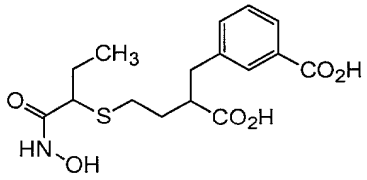
Compound	IC_{50}
 3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid	1060
 3-[2-Carboxy-5-(hydroxycarbamoyl-phenyl-methylsulfanyl)-pentyl]-benzoic acid	1120
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid	1530

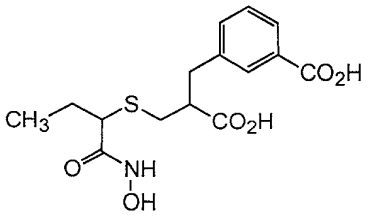
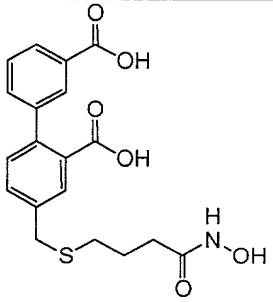
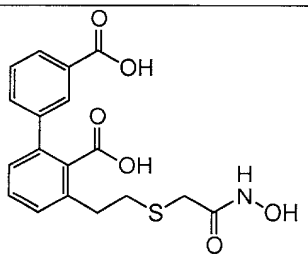
Compound	IC_{50}
 5-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-biphenyl-3-carboxylic acid	1690
 3-Bromo-5-(2-carboxy-5-hydroxycarbamoyl-methylsulfanylpentyl)-benzoic acid	2460
 3-Benzyloxy-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid	2570

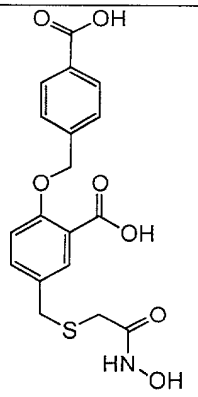
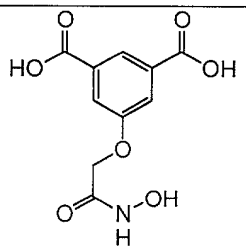
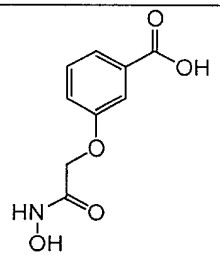
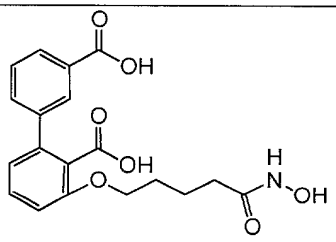
Compound	IC_{50}
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-2-methyl-propylsulfanyl)-pentyl]-benzoic acid	2800
 3-(2-Carboxy-3-hydroxycarbamoylmethyl-sulfanylpropyl)-benzoic acid	3070
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-5-phenoxy-benzoic acid	3250

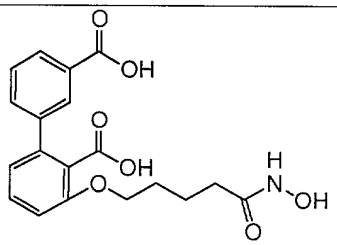
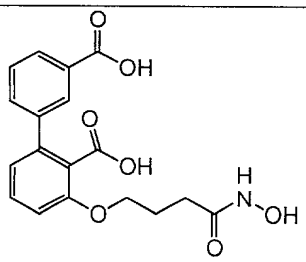
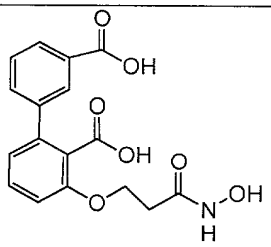
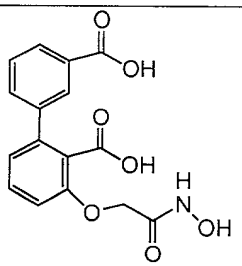
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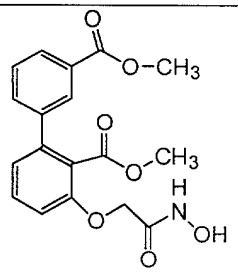
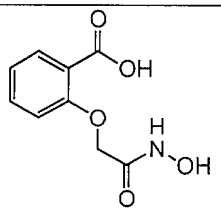
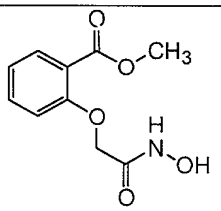
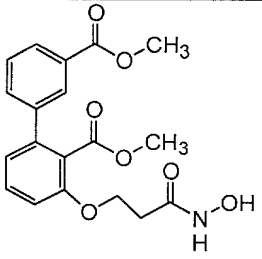
Compound	IC_{50}
 3-(2-Carboxy-6-hydroxycarbamoylmethyl-sulfanylhexyl)-benzoic acid	3330
 3-(2-Carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid	4900
 3-[2-Carboxy-3-(3-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid	6050
 3-[2-Carboxy-5-(4-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid	7580

Compound	IC_{50}
 3-{2-Carboxy-5-[(hydroxy-methyl-carbamoyl)-methylsulfanyl]-pentyl}-benzoic acid	14900
 3-*tert*-Butyl-5-[2-carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid	16000
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-4-chloro-benzoic acid	16000
 3-[2-Carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid	19000

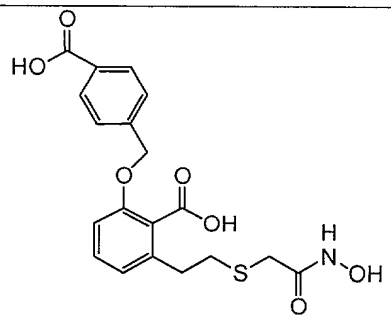
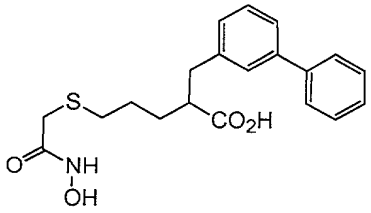
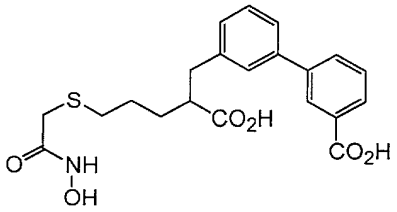
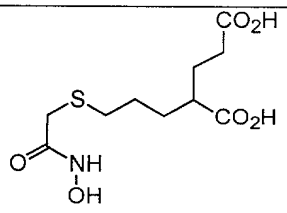
Compound	IC_{50}
 3-[2-Carboxy-3-(1-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid	41500
 4-(3-Hydroxycarbamoyl-propylsulfanylmethyl)-biphenyl-2,3'-dicarboxylic acid	10820
 3-(2-Hydroxycarbamoyl-methylsulfanyl-ethyl)-biphenyl-2,3'-dicarboxylic acid	255

Compound	IC_{50}
	1330
 5-Hydroxycarbamoylmethoxy-isophthalic acid	14800
 3-Hydroxycarbamoylmethoxy-benzoic acid	40800
 3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid	55.9

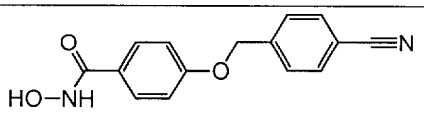
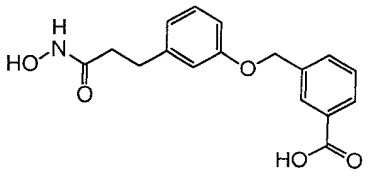
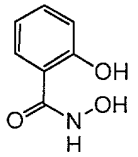
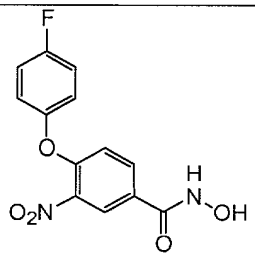
Compound	IC_{50}
 3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid	4800
 3-(3-Hydroxycarbamoyl-propoxy)-biphenyl-2,3'-dicarboxylic acid	9100
 3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid	14900
 3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid	27600

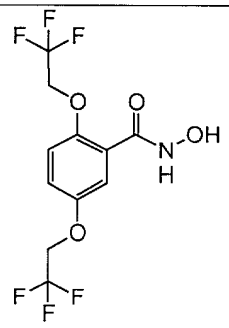
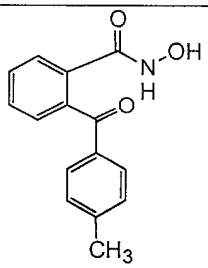
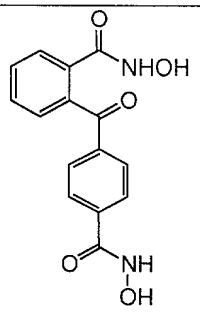
Compound	IC_{50}
 3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid dimethyl ester	100000
 2-Hydroxycarbamoylmethoxy-benzoic acid	100000
 2-Hydroxycarbamoylmethoxy-benzoic acid methyl ester	100000
 3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid dimethyl ester	100000

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Compound	IC_{50}
	14000
 2-Biphenyl-3-ylmethyl-5-hydroxy-carbamoylmethylsulfanyl-pentanoic acid	4210
 3'-(2-Carboxy-5-hydroxycarbamoylmethylsulfanyl-pentyl)-biphenyl-3-carboxylic acid	23500
 2-(3-Hydroxycarbamoyl-methylsulfanylpropyl)-pentanedioic acid	3000

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Compound	IC_{50}
 4-(4-Cyano-benzyloxy)-N-hydroxy-benzamide	14150
 3-[3-(2-Hydroxycarbamoyl-ethyl)-phenoxymethyl]-benzoic acid	92675
 2,N-Dihydroxy-benzamide	100000
 4-(4-Fluoro-phenoxy)-N-hydroxy-3-nitro-benzamide	100000

Compound	IC_{50}
 N-Hydroxy-2,5-bis-(2,2,2-trifluoro-ethoxy)-benzamide	100000
 N-Hydroxy-2-(4-methyl-benzoyl)-benzamide	100000
	100000

SUBSTITUTE SHEET (RULE 26)

EXAMPLE 13

Protective Effect of NAALADase Inhibitors in Experimental Rat Glaucoma

EXPERIMENTAL PROTOCOL

All experiments complied with the Association for

Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. 82 male Brown Norway rats (Rattus norvegicus), each weighing approximately 250 gm, were treated using procedures approved by the Animal Care Committee of the Johns Hopkins University School of Medicine. The rats were housed with a 12 hour light/12 hour dark cycle and fed *ad libitum*.

EXPERIMENTAL GLAUCOMA: Unilateral elevation of IOP was produced in 56 rats by microinjection of hypertonic saline into episcleral veins, following procedures described in Morrison, J. et al., *IOVS* (March 1998) 39:526-531. Beginning on the day of IOP elevation, the rats were treated daily with intraperitoneal injections of either a vehicle (23 rats with 50 mM HEPES-buffered saline) or a NAALADase inhibitor (11 rats with 10 mg/kg of 2-[[(2,3,4,5,6-pentafluorobenzyl)hydroxyphosphinyl]-methyl]pentanedioic acid ("Compound A") and 22 rats with 10 mg/kg of 2-(3-sulfanylpropyl)pentanedioic acid ("Compound B")). 11 saline treated rats, 11 Compound A treated rats and 11 Compound B treated rats were sacrificed at 8 weeks, and the remaining rats at 12 weeks, after initial IOP elevation.

OPTIC NERVE TRANSECTION: The optic nerve was transected unilaterally in 26 rats under intraperitoneal pentobarbital anesthesia. The conjunctiva was opened with scissors and the optic nerve exposed by traction on extraocular muscles. The transection was performed with

microscissors 5 mm posterior to the globe, with specific attention to avoidance of injury to major ocular blood vessels. Immediately after transection, the retina was examined ophthalmoscopically to assure that the retinal arterial blood supply was not disrupted. The conjunctiva was closed with absorbable suture and the eye dressed with antibiotic ointment. Beginning on the day of transection, the rats were treated daily with intraperitoneal injections of either a vehicle (9 rats with 50 mM HEPES-buffered saline) or a NAALADase inhibitor (8 rats with 10 mg/kg of Compound A and 9 rats with 10 mg/kg of Compound B). 5 saline treated rats, 3 Compound A treated rats and 4 Compound B treated rats were sacrificed at 2 weeks, and the remaining rats at 4 weeks, after transection.

OPTIC NERVE COUNTING: The rats were sacrificed by exsanguination under deep pentobarbital anesthesia. They were perfused through the heart with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, and the eyes with attached optic nerves were removed. A cross-section of the optic nerve from both experimental (glaucoma or transection) and control eyes was removed 1.5 mm posterior to the globe, 1 mm in thickness, and post-fixed in 2% osmium tetroxide in buffer. These were processed into epoxy resin, sectioned at 1 micron and stained with toluidine blue.

The area of the optic nerve cross-section was measured by outlining its outer border at 10x

magnification on an image analysis system (Universal Imaging Corp., Westchester, PA) with Synsys digital camera and Metamorph software. Three area measurements were taken and the mean value was determined. To measure the density and fiber diameter distributions, images were captured with a 100x phase contrast objective from 10 different areas of each nerve. These were edited to eliminate non-neural objects and the size of each axon internal to the myelin sheath (its minimum diameter) and the density of axons/square mm were calculated for each image and nerve. The mean density was multiplied by total nerve area to yield fiber number for each nerve. The total fiber number in glaucoma or transection nerves was compared to the normal, fellow eye of each rat to yield a percent loss value. The number of axons counted among the 10 images was an approximately 20% sample of the 80-90,000 axons in normal rat nerves. The person measuring axon number was masked to the protocol conducted on the nerves.

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RESULTS

EXPERIMENTAL GLAUCOMA: The mean fiber percent difference in the saline-treated, control rats was significantly lower in their glaucoma eyes compared to their normal eyes, with a mean ± standard errors from the mean (SEM) fiber loss of 14.44 ± 5.75 % (n = 11 rats; Table V) in the 8 week follow-up group, and 8.15 ± 7.84 % in the 12 week follow-up group (n = 12 rats; Table VI).

By contrast, there was no significant loss of fibers in either the 8 week or 12 week NAALADase inhibitor-treated rats. The mean percent fiber loss in each NAALADase inhibitor-treated group was statistically less than the loss in the saline-treated, control groups (at 8 weeks, p = 0.05 for Compound A and p = 0.02 for Compound B).

TABLE V

EXPERIMENTAL GLAUCOMA RESULTS

8 WEEK GROUP	N	IOP INTEGRAL DIFFERENCE ± SEM	FIBER NUMBER ± SEM	PERCENT DIFFERENCE ± SEM
Compound A	11	85 ± 37.5	79156 ± 2436 *	-1.82 ± 2.92
Compound B	11	116 ± 33.2	80785 ± 2121 **	-0.82 ± 2.97
Control	11	104 ± 26.4	68295 ± 4617	14.44 ± 5.75

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TABLE VI

EXPERIMENTAL GLAUCOMA RESULTS

12 WEEK GROUP	N	IOP INTEGRAL DIFFERENCE	FIBER NUMBER	PERCENT DIFFERENCE
Compound B	11	109 ± 45.2	90504 ± 1718	-3.21 ± 2.86
Control	12	158 ± 66.5	79827 ± 6783	8.15 ± 7.84

IOP Integral Difference = difference in intraocular pressure (IOP) exposure between glaucoma eye and normal eye in each rat (mm Hg -- days).

Percent Difference = mean percent difference in fiber number between glaucoma and normal eye in each rat (positive value indicates fewer fibers in the glaucoma eye).

Differences in IOP Integral Difference are not significant ($p > 0.05$).

Differences in Percent Difference between drug-treated and saline-treated, control rats at 8 weeks post insult are significant ($p = 0.05^*$ and $p = 0.02^{**}$).

OPTIC NERVE TRANSECTION: The experimental transection data suggest a slowing or rescue of ultimate RGC death in rats treated with NAALADase inhibitors at 2 weeks after transection. At 2 weeks after transection, both drug-treated groups had more remaining RGC axons than did the saline-treated, control group, judged either by absolute number of fibers or percent difference between transected eye and normal eye in each rat (TABLE VII). Rats treated with Compound A and Compound B had, respectively, 3 times and twice as many remaining axons as the saline-treated rats. All or nearly all RGC die within the first 2 months after transection, regardless of any pharmacological treatment. Thus, by 4 weeks after transection, more than 80% of RGC axons were gone in all groups (TABLE VIII). At 4 weeks after transection, there were no significant differences between the drug-treated rats and the saline-treated rats.

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TABLE VII

OPTIC NERVE TRANSECTION

2 WEEKS SURVIVAL	N	FIBER NUMBER ± SEM	PERCENT DIFFERENCE ± SEM
Compound A	3	26,426 ± 13,293	65.3 ± 17.8
Compound B	4	19,550 ± 5,091	75.3 ± 6.6
Control	5	8,220 ± 4,668	90.2 ± 5.35

TABLE VIII

OPTIC NERVE TRANSECTION

4 WEEKS SURVIVAL	N	FIBER NUMBER ± SEM	PERCENT DIFFERENCE ± SEM
Compound A	5	13,599 ± 3,519	82.4 ± 4.0
Compound B	5	5,162 ± 2,509	93.4 ± 3.1
Control	4	10,449 ± 3,648	86.9 ± 4.7

Percent Difference = mean percent difference in fiber number between glaucoma and normal eye in each rat (positive value indicates fewer fibers in the glaucoma eye).

Differences in Percent Difference between drug-treated and saline-treated, control rats are not statistically significant ($p = 0.05$).

EXAMPLE 14

Efficacy of NAALADase Inhibitors in Treating Retinal Disorders

Four (4) groups of rats received daily insulin injections to maintain their glucose levels at about 350 mg/dl. Starting at the onset of hyperglycemia, NAALADase inhibitor 2-(3-sulfanylpropyl)-pentanedioic acid was administered daily for 6 months to one group of BB/W rats at a dose of 10 mg/kg and to a second group of BB/W rats at a dose of 30 mg/kg. A third group of BB/W rats and a fourth group of non-diabetic rats received daily vehicle treatment (50 mM Hepes buffered saline).

After six (6) months of NAALADase inhibitor or vehicle treatment, the rats were sacrificed and their eyes were removed. From each rat, one eye was processed for elastase digest while the other eye was processed for transmission electron microscopy (TEM) and basement membrane (BM) thickness.

Elastase Digests

Retinal digests were prepared using elastase on retinas as described in Layer, N., *Invest Ophthalmol Vis Sci* (1993) 34:2097. Eyes were removed from recently killed BB/W rats (n=25) and age-matched transgenic controls (n=10). The retinas (n=35) were fixed at room temperature by immersing the whole eye (slit at limbus) in 4% (w/v) paraformaldehyde in 50 mmol/L Na-K phosphate buffer with 8% sucrose. The fixed retinas were rinsed in deionized water and were incubated for 3 minutes in a

37°C agitating water bath in 40 units/mL elastase in Na-K phosphate buffer with 150 mmol/L NaCl and 5 mmol/L ethylenediamine tetraacetic acid (EDTA), pH 6.5. The tissues were washed overnight in 100 mmol/L Tris-HCL (pH 8.5) and then transferred to deionized water for removal of the loosened vitreous and digested neural elements by gentle agitation using the sides of closed forceps and the sides and ends of very fine brushes. After all loose tissues were removed, the retinas were incubated once more in fresh enzyme for 3 minutes and then subjected to a second overnight wash at room temperature in Tns-HCl buffer. On the third day, the retinas were again transferred to deionized water for additional removal of digested neural elements. The vascular network that was completely free of nonvascular elements was mounted flat by flotation in Ca^{2+} and Mg^{2+} free Dulbecco's PBS on siliconized slides (#S1308, Oncor, Gaithersburg, MD). After air drying in a dust free environment, the mounts of the retinal microvasculature were stained using periodic acid Schiff reaction and hematoxylin counterstaining, as described in Luna, L., ed. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology* (1968) McGraw-Hill, New York, NY. The preparations were then examined by light microscopy and photographed.

Endothelial/Pericyte (E/P) Ratios

The stained and intact retinal whole mounts were coded and subsequent counting was done masked, as

described in Cuthbertson, R., *Invest Ophthalmol Vis Sci.* (1986) 27:1659-1664).

Ten fields at x100 magnification were counted for endothelial and pericyte cells using previously described morphologic criteria (see Kuwabara, T., *Arch Ophthalmol.* (1960) 64:904-911). In every sample, at least 200 cells were counted from the mid zone of the retina. Mean values for endothelial cell/pericyte (E/P) ratios were initially calculated in 3 retinas from each of the four (4) groups of rats.

Evaluation of BM Thickness

Each eye was fixed in 4% glutaraldehyde and dissected free of sclera and choroids, then trimmed and postfixed in 1% osmium tetroxide. After dehydration and embedding, thin sections were stained with uranyl acetate and lead citrate. Initially, BM thickness of retinal capillaries from 3 non-diabetic rats receiving vehicle, 3 diabetic animals receiving 10 mg/kg 2-(3-sulfanylpropyl)-pentanedioic acid, and 3 diabetic rats receiving 30 mg/kg 2-(3-sulfanylpropyl)-pentanedioic acid were compared with 3 diabetic rats receiving a vehicle. At least 10 capillaries per eye from the inner nuclear and plexiform layers were photographed at a magnification of 10,000x. Exact magnification was determined for each set of negatives with a 28,800 line/inch calibration grid. Negatives were enlarged 3x. Measurements, to the nearest 0.25 mm, were made of the basement membrane surrounding the endothelial cell and were taken perpendicular to the

plane of the basement membrane, as described in Bendayan, M., *J. Electron Microsc Techn* (1984) 1:243-270; and Gunderson, *J. Microscopy* (1980) 121:65-73). At least 20 measurements were taken for each capillary and the BM thickness was expressed as an average of 20 measurements.

Statistical Analysis

Statistical analysis for comparison among groups was performed using one way analysis of variance (ANOVA) and Student's t test. Significance was defined as a value of $p<0.05$. Values were reported as mean ± standard errors from the mean (SEM), unless otherwise noted.

Results of Elastase Digest Preparations and E/P ratios

In intact whole mounts of retinal digests the endothelial cell nuclei, seen medially within the vessel wall, were large, oval, pale staining and protruded lumenally. Pericyte nuclei, seen more laterally, were dark staining, small, round and protruded prominently away from the vessel wall. E/P counts were taken from mid zones of the retinas. The attached figures show 27,000x magnified photographs of retinal blood vessels from a control, non-diabetic rat (FIG. 1), from a control, diabetic rat after six (6) months of treatment with a vehicle (FIG. 2), and from a diabetic rat after six (6) months of treatment with NAALADase inhibitor 2-(3-sulfanylpropyl)-pentanedioic acid (FIG. 3). In the figures, "BM" refers to basement membrane, "EC" refers to endothelial cell, and "L" refers to vessel lumen.

NAALADase inhibition had no effect on blood glucose or body weight. Six month high dose (30 mg/kg) treatment with 2-(3-sulfanylpropyl)-pentanedioic acid resulted in a 29.0% reduction in BM thickness (diabetic vehicle = 101.0 ± 14.81 nm and diabetic $NAALADase_{30}$ = 71.7 ± 4.07 nm), while treatment with the low dose resulted in an 18.5% decrease in BM thickness ($NAALADase_{10}$ = 82.3 ± 4.07 nm). This was accompanied by a 37 % reduction of E/P ratios in rats treated with the high dose 2-(3-sulfanylpropyl)-pentanedioic acid (diabetic vehicle = 3.0 ± 0.3 and $NAALADase_{30}$ = 1.9 ± 0.4), while treatment with low dose resulted in a 20% reduction of the same cell ratios ($NAALADase_{10}$ =2.4 ± 0.5). See TABLE III.

TABLE III

RAT GROUP	BM THICKNESS (nm) ± SD (n = 3)	E/P RATIO (n = 8-10)
NON-DIABETIC CONTROLS	56.3 ± 4.78	1.7 ± 0.07
DIABETIC VEHICLE	101 ± 14.81	3.0 ± 0.3
DIABETIC 30 MG/KG NAAALADASE INHIBITOR	71.7 ± 4.07	1.9 ± 0.4
DIABETIC 10 MG/KG NAALADASE INHIBITOR	82.3 ± 4.07	2.4 ± 0.5

Conclusions

The BB/W rats demonstrated an early change typically associated with diabetic retinopathy (pericyte loss and basement membrane thickening) but did not show significant numbers of microanuerysms also typical of diabetic retinopathy or areas of acellular capillaries usually seen in more advanced disease. The retinopathy observed in BB/W has been previously characterized in Chakrabarti, *Diabetes* (1989) 38:1181-1186.

The results show that treatment with a NAALADase inhibitor causes improvement in retinal pathology of diabetic rats. Specifically, the NAALADase inhibitor prevented pericyte loss and basement membrane thickening in retinal vessels.

All publications, patents and patent applications identified above are herein incorporated by reference, as though set forth herein in full.

The invention being thus described, it will be apparent to those skilled in the art that the same may be varied in many ways without departing from the spirit and scope of the invention. Such variations are included within the scope of the following claims.